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AIM AND SCOPE

World Journal of Hepatology (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

WJH covers topics concerning liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, biliary tract disease, autoimmune disease, cholestatic and biliary disease, transplantation, genetics, epidemiology, microbiology, molecular and cell biology, nutrition, geriatric and pediatric hepatology, diagnosis and screening, endoscopy, imaging, and advanced technology. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

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TOPIC HIGHLIGHT

WJH 6th Anniversary Special Issues (1): Management of hepatocellular carcinoma

Management of "very early" hepatocellular carcinoma on cirrhotic patients

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Abstract

Due to the advances in screening of cirrhotic patients, hepatocellular carcinoma (HCC) is being diagnosed in earlier stages. For this reason the number of patients diagnosed of very early HCC (single tumors ≤ 2 cm) is continuously increasing. Once a patient has been diagnosed with this condition, treatment strategies include liver resection, local therapies or liver transplantation. The decision on which therapy should the patient undergo depends on the general patients performance status and liver disease. Anyway, even in patients with similar conditions, the best treatment offer is debatable. In this review we analyze the state of the art on the management of very early HCC on cirrhotic patients to address the best treatment strategy for this patient population.

Key words: Hepatocellular carcinoma; Very early; Liver resection; Liver transplantation; Local therapies

Core tip: Very early hepatocellular carcinoma patients are deemed too early for liver transplantation candidacy, known as the best treatment regarding long-term survival and tumor recurrence. Strategies as surgical resection and radiofrequency ablation have gained popularity. Although resection is considered as the first line of treatment, recent studies claim equal results with ablation techniques. Ablation used as a test of time in patients who remain candidates for liver transplantation is attractive. In this review we will analyze in detail the novel strategy repertoire used in the management of these patients.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary malignancy of the liver, the sixth most common cancer worldwide and the third largest cause of cancer-related deaths[1-3]. The incidence of HCC is increasing in Europe and in the United States[4] and it is currently the leading cause of death among cirrhotic patients[5].

The management of these tumors has significantly improved over the last few years due to a better knowledge of the natural history of the malignancy and the development of staging systems. One of the most reliable and widely adopted methods for staging HCC is the Barcelona Clinic Liver Cancer (BCLC) system[6], that stratifies patients according to the characteristics of the tumor, underlying liver disease and performance status. According to this system, the presence of an asymptomatic single nodule ≤ 2 cm, in the absence of vascular invasion or extrahepatic disease, has been defined as very early stage HCC[7-9].

In recent years, thanks to surveillance programs on the cirrhotic population, more patients are being diagnosed with very early HCC[9-11].

There are basically three potential curative modalities of treatment for patients diagnosed of very early HCC: Liver resection (LR), liver transplantation (LT) and radiofrequency ablation (RFA). Although these patients show excellent outcome in terms of survival and recurrence[12] compared to those with more advanced tumors, the debate regarding what is the best treatment option in that scenario still remains[3,13].

Our aim is to review the current management of very early HCC on cirrhotic patients.

DIAGNOSIS OF VERY EARLY HCC

Hepatocellular carcinoma is frequently diagnosed by imaging criteria based on the contrast enhancement pattern. Early detection by surveillance is the only way to diagnose HCC when curative treatments are feasible, being the optimal profile for this endpoint very early HCC[9,10]. Intense contrast uptake in the arterial phase followed by contrast washout in the venous phase, both on computed tomography or magnetic resonance, is considered diagnostic for HCC > 1 cm[9,14]. Nevertheless, on cirrhotic patients, small lesions may be misdiagnosed as being HCC and can in fact be intrahepatic cholangiocarcinomas (iCCA) or mixed hepatocellular-cholangiocarcinomas (HCC-CC), being their frequency much lower[15,16].

If the lesion does not show the typical HCC pattern on imaging, biopsy is mandatory[10]. A prospective study including 89 cases with liver nodules between 0.5 and 2 cm reported that non-invasive criteria had a sensitivity of 30%, being necessary a biopsy for their diagnosis[17]. However, pathological diagnosis is particularly complex for nodules < 2 cm, being difficult the distinction between high-grade dysplastic nodules, intrahepatic cholangiocarcinomas (iCCA) and HCC[17]. It is currently considered that a positive tumor biopsy is clinically useful to diagnose an HCC, while a negative biopsy cannot rule out malignancy[18,19].

Anyway, despite the misdiagnosis of small nodules, current data has shown interesting results on the outcome of patients diagnosed of "very early" iCCA and HCC-CC at pathology. These studies demonstrated excellent post-transplant survival for patients with such tumors on pathology. Nevertheless, future studies must be conducted to confirm these results[15,16].

LIVER RESECTION

Liver resection constitutes the first-line treatment option for patients with very early HCC and compensated cirrhosis in most centers[3,11,20]. As indicated by the BCLC, this is especially true when patients are potential candidates for LT[21,22] as we will analyze later in detail.

Partial LR in cirrhotic patients must be addressed under two contradictory principles: to be a curative resection and to preserve as much liver parenchyma volume as possible to avoid postoperative liver failure[1]. Thanks to recent advances in surgical technique and immediate postoperative care, the modern standards for resection of HCC in cirrhotic patients have improved and include a perioperative mortality less than 1%, blood transfusion requirements below 10% and 5-year survival rates of at least 50%[20]. Anyway, major resections are not recommended even in compensated cirrhotic patients because of the risk of post operative liver failure due to an insufficient remnant liver, which can lead to death[9]. Nevertheless and thanks to the advance in several techniques such as portal vein embolization, some groups perform major hepatectomies for HCC after portal vein embolization if there is a sufficient growth of the liver remnant[23,24].

The discussion between anatomic *vs* non anatomic resection still remains. Most studies defend anatomic resection as a method to avoid or ameliorate local recurrence[25,26]. Other studies have not been able to confirm this[27]. If the invasive phenotype is minor, as in the case of very early HCC, the spread beyond the segment may be low and anatomic resection may provide a benefit[9]. Basically the recommendation would be to perform an anatomic resection whenever possible and safe.

One of the main contraindications for LR in cirrhotic patients is the presence of portal hypertension. The BCLC group identified the absence of clinically relevant portal hypertension and normal bilirubin as the key variables to make a safe selection of candidates for LR. An hepatic venous pressure gradient ≥ 10 mmHg was shown to be a predictor of unresolved hepatic decompensation and, consequently, of poor long-term outcome in Child-Pugh A cirrhotic patients after surgery[14,28]. The presence of esophageal varices detectable at endoscopy, splenomegaly and/or a platelet count less than 100000 were considered indirect signs of portal hypertension[29]. The value of portal hypertension assessment in predicting prognosis has been confirmed also by japanese groups[30]. However, some authors have reported good results for patients resected with portal hypertension. Cucchetti *et al*[31], found in 2009 after one-to-one matching, that the only predictors of postoperative liver failure were model of end-stage liver disease (MELD) score and the extent of hepatectomy and so, did not found portal hypertension as a risk factor[31]. Ruzzenente *et al*[32], also concluded that portal hypertension is not an absolute contraindication to liver resection in Child-Pugh class A cirrhotic patients but noted a worse survival in patients who were resected two or more segments if portal hypertension was present probably showing the higher risk of more extended hepatectomies in the cirrhotic population[32]. Anyhow, most centers would only perform LR if portal hypertension is not present, and so, despite the results of some retrospective studies[33], prospective multicenter studies should be conducted to assess the safety of LR in the presence of portal hypertension. Even though the presence of portal hypertension may not be considered an absolute contraindication for LR, it will significantly affect patients early and late outcome after resection.

One of the principal advantages of LR over other

treatments like local therapies is the pathological examination of resected tumors. Indeed, this may represent a very useful tool to predict the risk of recurrence and to select patients with HCC who are likely to obtain the maximum benefit from LT[1,34]. Accordingly, the BCLC recommend LR in cirrhotic patients with very early HCC who are candidates for LT. Histological features on the LR specimen have been proposed as a guide for selection of LT candidates and as a tool for optimization of the donor pool. In selected cases and according to characteristics in specimen aggressiveness, resection may be considered as a bridge to transplantation[35].

Cillo *et al*[36] reported tumor differentiation as a direct marker of biologic tumor aggressiveness, providing interesting information about the risk of recurrence[36].

The BCLC and other groups have proposed a policy of listing patients for LT without evident HCC based on pathological risk of recurrence after resection, characterized by the presence of vascular invasion and/or satellitosis. They have given the name "ab initio" indication, also known as "de principe" LT[34,36-39]. Both parameters, presence of microvascular invasion and additional nodules, could be used to stratify resected patients in two categories: patients with low risk of recurrence and patients with high risk of recurrence[30,40]. The rate of microvascular invasion increases according to the tumor size and it is present in 20%-25% of HCC less than 2 cm[14,41]. Sala *et al*[34] reported in 2004 the efficacy of this strategy in 6 patients who were transplanted after being diagnosed with high risk recurrence (according to gross and microscopic examination after LR) with good results[34]. Scatton *et al*[35] published a retrospective cohort study in 2008, in which de principe LT was proposed to 6 patients because of poor prognosis histological findings on the resected specimen, reporting that all these patients were alive at the time of publication, with a mean follow-up of 55 mo[35]. On the other hand, other authors have proposed that patients who exceed Milan criteria and present poor histological findings at the time of resection, should be precluded from LT because of the high risk of recurrence, while patients exceeding Milan criteria but with good histological prognostic factors may benefit from de principe LT[34,35].

Some recent studies have proposed a molecular signature to define the level of risk due to the oncogenicity of the cirrhotic liver. This concept still has to be validated in clinical practice[9], but looks very promising.

Recurrence after LR

The main problem after LR for HCC is the high rate of tumor recurrence[1,13,42]. There are several reports indicating that the 5-year recurrence rate is up to 80%-100%[43-46].

The most common site of post-resection recurrence is the remaining cirrhotic liver[47], as the persistent underlying liver disease (main risk factor for the development of HCC) is associated with high rates of intrahepatic recurrence[48]. Basically, two types of tumor recurrence after LR have been described: local recurrence, which usually happens in the first 2 years after resection and may be the result of inadequate R1 resection or secondary to the progression of microscopic vascular invasion and "de novo" recurrence, which happens more than 2 years after resection and constitutes the development of a new tumor due to the presence of underlying cirrhosis[49].

Patients with very early HCC can achieve 5-year survival rates around 90% after resection and extremely low 3-year recurrence rates have been described (around 8%)[3,50]. Other published studies reported similar survival but the disease free survival was around 40% at five years[50,51]. The largest retrospective experience on the outcomes of LR in very early HCC was reported by Ikai *et al*[52] analyzing 2320 patients and finding a 3- and 5-year survival of 84% and 66% respectively. Lee *et al*[53] also reported similar outcomes, with a 3-year survival of 82.5%. None of these studies specified on the recurrence rate after very early HCC.

Treatment of HCC recurrence after LR is currently based on several strategies that include the use of antineoplasic drugs, RFA, chemoembolization, alcoholization, re-resection and liver transplantation; being the most curative therapies the last two[54].

Re-resection: The applicability of re-resection will be determined by the patient general performance status and liver function at the time of recurrence. Some authors have described a low applicability rate (10%-25%) for re-resection and argue that it should ideally be restricted to "*de novo*" cases and not "local recurrences"[55,56]. Several studies have demonstrated good results after re-resection. Poon *et al*[57] reported a 5-year survival rate after re-hepatectomy of 69.3% and Sugimachi *et al*[56] concluded in another study that despite patients with recurrence treated with re-hepatectomy having a better prognosis compared to patients with recurrence who did not have a repeat hepatectomy, re-resection must be performed in selected patients[56]. Anyhow, whenever possible, re-resection should be considered at the time of recurrence and analyzed in a patient to patients basis.

Salvage LT: As previously stated, LR constitutes the first-line therapy for very early HCC on potential candidates for LT with compensated liver cirrhosis. In these regards, surgeons may have in mind that patients can be transplanted at the time of recurrence[58]. This strategy of secondary LT is called salvage transplantation[27]. Poon *et al*[59] published that 80% of patients with recurrence after a LR for HCC remain eligible for LT. Although some authors have published similar results regarding the applicability of salvage transplantation[60], in clinical practice the real applicability of this policy is low, only 10%-20% of cases, as it has been shown in several studies[61,62]. In a previous study from our department, we reported a series of 17 potential candidates for salvage LT, but could only be performed in 6 patients. Age at the time of recurrence was the main reason for contraindicating LT. In spite of that, we found that results of salvage transplantation were similar to those of primary LT[63]. The main problem with this strategy is related to a high drop out of resected patients

from LT, due to a non-transplantable recurrence, tumor progression during the waiting time or life-threatening complication of underlying cirrhosis, and so, the feasibility of salvage transplantation will be closely related to a strict surveillance after resection and the consequent early diagnosis of intrahepatic recurrence[62]. Although there is conflicting results when comparing primary LT and salvage transplantation and there is a concern on the higher risk of complications in patients that receive a transplant after LR, most studies showed no differences when comparing biliary leaks, vascular complications, re-operation or re-transplantation rates[64,65]. Nevertheless, operative mortality and bleeding have been described to be significantly higher after salvage transplantation in some series[62]. As no randomized controlled trials are feasible in this regard, and methodological pitfalls of current data exist, comparable outcomes are still a matter of debate.

A determining factor when including a patient in the waiting list for salvage transplantation is the time from LR to recurrence. Early recurrence (before 1 year) after LR has been found to be a risk factor for poor outcome after transplant probably indicating the tumors aggressiveness[54].

In patients with very early HCC or small single tumors (< 3 cm), salvage transplantation may be more applicable as recurrence of these tumors can be more limited. This may explain the excellent 10-year survival when comparing patients diagnosed with a very early HCC that are transplanted or resected[13,66].

LOCAL THERAPIES

In the last decade RFA has become one of the standard treatments of very early HCC on cirrhotics[67,68]. This treatment can be included basically in 2 strategies: intended as a definitive curative treatment or as a bridge to LT.

According to the European Association for the Study of the Liver (EASL) and the European Organization for Research and Treatment of Cancer, percutaneous ethanol injection (PEI) and RFA are considered as the standard of care for HCC patients with very early HCC not suitable for surgery[16]. According to the American Association for the Study of Liver Diseases (AASLD), PEI was initially suggested as the standard against which any percutaneous therapy should be compared[22]. However, recent studies demonstrate that RFA has better local control for HCC > 2 cm. In tumors < 2 cm RFA and PEI have equal results[69]. Patients with very early HCC do not afford any priority points on the waiting list and generally have low MELD scores, the probability of attracting an organ is very low[70,71]. Accordingly the debate arises on to what is the best option for these patients: immediate ablation or wait until the tumor grows and then patients afford exception points and have real options of attracting an organ[71].

Several studies have shown the efficacy of local therapies on very early HCC[72-76]. Sala *et al*[77] reported a 50% survival at 5 years in Child A patients and treatment response in 70% of nodules < 3 cm and 50% in nodules > 3 cm or multi-nodularity, achieving results that could almost be equal to surgical resection in selected patients. Livraghi *et al*[78], conducted a multicenter study enrolling cirrhotic patients with HCC < 2 cm undergoing RFA, of whom 46% where initial candidates for surgery. The estimated 3-year and 5-year survival rates were 76% and 55%, respectively and 65% in the subgroup that could potentially have been resected; thus achieving a 5-year survival rate similar to that achieved after surgical resection[78]. The advantages of RFA over surgery were less invasiveness, complications, hospital stay, blood transfusions and treatment costs. N'Kontchou *et al*[79] evaluated long term outcomes of RFA as the first line treatment and prognostic variables in patients with early-stage HCC defined as tumors < 5 cm and less than 3 tumors. They had a complete radiological ablation in 94.7% of their cohort with estimated overall 3 and 5-year survival rates of 60% and 40% respectively. The estimated 3 and 5-year recurrence-free survival rates were 37% and 18% respectively, with a median recurrence-free survival of 23 mo. The size of the tumor was found to be a predictor of local recurrence, but not of overall or tumor-free survival rates. Recurrences where limited and ablated by additional RFA sessions[80]. RFA has been suggested, according to these studies, as an adequate treatment for small HCC, having less side effects and in case of recurrence, multiple RFA sessions could control the disease without comprising survival.

As previously stated, RFA has emerged as the first line treatment for patients with very early HCC non candidates for LT and as a curative-intent treatment for HCC in some centers, as patients will not be afforded with exception points and then wait very long times for a graft[80-82]. The most important limiting factor to this strategy includes post RFA recurrence of 50%-80% at 5 years. Emergence of new tumors rather than local tumor progression seems to be responsible for these results[77-80,83]. A two-step strategy comprises performing RFA to LT candidates with HCC, leaving transplantation as definite therapy only if recurrence or liver failure occurs. A previous "test of time" would identify those patients with aggressive tumor biology who would carry a high risk of recurrence post LT, thus optimizing the scarce resource of organ donors and reducing the burden of HCC patients on the waiting list[4,79]. As stated in a recent editorial by Yao, patients selected for this strategy should be those who have a high probability of long-term cure with a low risk of recurrence[68]. Limitations to this strategy, include the uncertainty for those patients who do not remain as candidates for salvage transplantation according to the size and number of recurrent tumors. Tsuchiya *et al*[84] published a retrospective analysis of 323 patients undergoing RFA as initial treatment of which 60% of patients suffered recurrence beyond transplantation criteria and only 30% of these patients were eligible for salvage LT. Predictors of HCC recurrence were AFP > 100 ng/mL, tumor size > 2 cm, and early recurrence within 1 year[84]. This has raised the question if the "ablate and wait" strategy may result in a percentage of patients with recur-

rence out of transplantable criteria and then loose the opportunity for LT. N'Kontchou *et al*[79] reported promising results with the "two step" strategy, using RFA as first line treatment in patients eligible for LT. The 3- and 5-year overall recurrence rates were 50% and 58%, respectively. For Child A patients, survival rates at 5 years were comparable to that of patients who were offered LT as first line therapy[79].

We believe that the best candidates for RFA as first line treatment would be those Child A patients with solitary lesions ⩽ 2 cm who fail to recruit enough exception points on the waiting list as this patients achieve the best long term survival and best complete initial response[68,78,79]. Anyway, these patients should undergo strict follow-up to diagnose recurrence in an early manner.

LIVER RESECTION *VS* RADIOFREQUENCY ABLATION

According to the clinical guidelines by the AASLD and the EASL, surgical resection is the first line treatment for patients with small solitary HCC Child A cirrhosis and no portal hypertension[22]. RFA is an optional treatment for small HCC, obtaining similar results regarding long-term survival compared to surgical resection. Several meta-analysis have tried to assess the advantages and disadvantages of RFA compared to surgical resection. Conflicting data has been obtained from these studies regarding overall survival and disease free survival due to the retrospective nature of the studies involving heterogeneous cohorts. Moreover most of these studies have not analyzed patients with very early HCC in detail[85,86].

Some randomized controlled trials have been performed to assess this issue; none of them strictly analyzes the subgroup of patients with very early HCC. Huang *et al*[87] assessed, in an intention to treat analysis, overall survival, recurrence free survival and overall recurrence comparing 115 RFA patients with 115 surgical resected patients, (both groups had tumors within the Milan criteria). After a 5-year follow-up, overall survival rates of RFA and surgical resection were 54.78% and 75.65%, respectively. Overall survival and recurrence-free survival were significantly higher in the surgical resection group than in the RFA group. Once stratifying by tumor size, surgical resection still offered an advantage over RFA in patients with early HCC (defined as tumors < 3 cm). RFA had an advantage in terms of less hospital stay and less adverse events[87]. Feng *et al*[88] evaluated survival and recurrence undergoing a randomized controlled trial in an intention to treat basis comparing RFA *vs* surgical resection. Early HCC was defined as tumors with a maximum diameter of 4 cm and up to 2 nodules. The 1, 2, and 3-year overall survival rates were 96.0%, 87.6%, 74.8% and 93.1%, 83.1%, 67.2% for the surgical resection and RFA groups, respectively. Recurrence-free survival was 90.6%, 76.7%, 61.1% for the surgical resection group and 86.2%, 66.6%, 49.6% for the RFA group. No significant differences were seen between the two groups regarding overall and recurrence-free survival. No stratified analysis regarding tumor size and outcomes on both groups was presented. Again, patients that underwent RFA had less hospitalization stay and less blood transfusion rates. Chen *et al*[89] evaluated a cohort of 90 patients who underwent surgical resection compared to 90 patients who underwent RFA. Early HCC was defined as a solitary lesion less than 5 cm. There were no differences in the overall and disease free survival rates. Stratified analyses of both therapeutic interventions for lesions less than 3 cm revealed no differences[89]. The information from the randomized controlled trials on the outcomes of RFA *vs* LR for very early HCC is not clear and the outcomes comparing these therapies still require further investigations.

On the other hand, several observational retrospective studies do make emphasis on very early HCC and outcomes after RFA and surgical resection, however, they lack randomization and may be biased by covariate distribution[90,91]. Hung *et al*[90] analyzed a cohort of patients with very early HCC that included 66 patients in the RFA group and 50 in the surgical resection group. There were no statistically significant differences in terms of overall survival and recurrence but both groups were heterogeneous[90]. Peng *et al*[92] compared retrospectively the effects of RFA and surgical resection in patients with resectable HCC < 2 cm. Seventy-one patients treated with RFA were compared with 74 surgically treated. Overall survival rates at 1, 3, and 5-years were 98.5%, 87.7%, and 71.9% in the RFA group compared to 90.5%, 70.9%, and 62.1% in the surgical resection group. No differences were found regarding disease-free survival between groups. The main problem with this study was its retrospective nature that leads to several selection bias[92]. Wang *et al*[51] compared in a cohort of very early HCC patients, 51 patients undergoing RFA *vs* 91 patients undergoing surgical resection. There was no significant difference in overall survival between groups; however, patients treated by surgical resection had a better disease free survival than those in the RFA group. They suggested that surgical resection should be the preferred modality in very early HCC when liver transplantation is not feasible[51]. Finally, Takayama *et al*[91] published a large Japanese multicenter study analyzing RFA *vs* surgical resection in a cohort of 2550 patients. Basically half of the patients were treated with RFA and half were operated. Disease free survival was significantly better in the surgical resection group compared to RFA. Overall survival in both groups showed no differences. Again, due to the retrospective nature of their study, several selection bias were found. Percutaneous ablation was more prominent in Child B patients who had more hepatic dysfunction compared to those who underwent resection[91].

Surgical resection continues to be the first line treatment for patients with early HCC suitable for surgical therapy; however, many patients cannot be offered resection because of liver dysfunction at the time of diagnosis. RFA seems to be a suitable modality of treatment for these patients, achieving similar results regarding disease free survival and overall survival according to the available

information. The decision on whether to perform RFA or resection of patients with very early HCC will depend on the type of resection required, the general performance status of the patients and their liver function.

LIVER TRANSPLANTATION

Liver transplantation is accepted as the best treatment modality for HCC, as it efficiently removes the tumor within the liver and the remaining oncogenic cirrhotic tissue caused by the underlying pathology[9,93]. Despite the efforts for assuring transplantability for HCC patients according to the Milan Criteria and expansion of these parameters by the University of California, San Francisco criteria, scarcity of organ donors and the increased number of patients on the waiting list renders patients to undergo other treatment modalities[94-98]. According to the principle of utility, ablation and resection in tumors ⩽ 2 cm avoids futile transplantation in these patients[75,95,99,100].

In patients with tumors ⩽ 2 cm LT is not considered as first line treatment as these patients are deemed "too early" for transplantation and may not be listed with exception points. Three strategies for management may be considered; RFA, surgical resection or waiting for tumor progression with subsequent listing once the tumor exceeds 2 cm.

The wait and not ablate strategy considers waiting for tumors to exceed 2 cm and then seek exception points for LT. With this strategy 9% of patients progress from T1 to directly beyond T2 before listing, drop-out rates once on the waiting list account for 7%-10% of patients, and 3-year survival rates with transplantation achieve 75%[70,71].

Although LT is the best strategy for the treatment of HCC regarding survival and recurrence, in the setting of very early HCC, RFA and surgical resection continue to be first line treatments. The wait and not ablate strategy seems to have good results, however, robust evidence is still lacking as to how and when to apply it considering patients eligible for other ablative techniques[70].

Nowadays, LT remains as a second line treatment for patients with very early HCC and low MELD scores. The main benefit of LT is the treatment of recurrence after LR or RFA. Anyhow, this statement must be taken with caution as some patients may loose their opportunity to be transplanted if recurrence exceeds each centers transplantation criteria.

LIVER RESECTION *VS* LIVER TRANSPLANTATION

Many publications have compared the results of LR and LT for HCC, in general, they have found similar patient survival with better disease-free survival in patients undergoing LT[48,101-105]. However, there are not many publications that focus on the outcomes of very early HCC.

Bismuth *et al*[103] published in a retrospective study that in case of small uninodular or binodular tumors smaller than 3 cm, LT had much better results than resection, showing a disease free survival of 83% *vs* 18% in resected patients[103].

Cha *et al*[101] concluded that partial hepatectomy in patients with early HCC who are otherwise eligible for LT can be performed with minimal morbidity and can achieve comparable 5-year survival to that reported for LT. They stated that resection should be considered the standard therapy for patients with HCC who have an adequate liver reserve[101].

Another publication by Margarit *et al*[63], comparing outcomes of LR and LT in a retrospective study with 259 patients, found no significant differences in overall actuarial survival, with a median survival of 85 mo in both groups. They reported that HCC recurrence was significantly higher after LR (59%) than LT (11%). However, this study included all the patients who presented tumors smaller than 5 cm and the mean tumor size was 3 cm[63].

The publications listed above did not report longer 5-year follow-up, nor did they distinguish between very early and early HCC.

There are two studies (recently published) that tried to assess the long-term outcome (10 years) for patients resected and transplanted. Adam *et al*[62] compared results of HCC < 5 cm after LR or LT under the policy to prioritize Child A patients with peripheral lesions for resection rather than transplantation, finding better results for transplanted patients. For single HCC smaller than 3 cm, they found that LR achieved comparable 10-year overall survival[13]. In another study published by our department, the outcomes were similar to Adam's paper. We compared patients with HCC < 5 cm who underwent LT or LR, finding a higher tumor recurrence rate in resected patients and better survival in patients who were transplanted. However, when we analyzed those tumors < 2 cm, no significant differences were observed in 1-, 5- and 10-year survival between the two groups[66].

The good outcomes of these publications could be justified because the recurrences in very early HCC are easier to treat, whether by re-resection or especially by salvage transplantation, allowing LR to be the treatment of choice for these tumors.

CONCLUSION

The best approach for cirrhotic patients diagnosed of very early HCC is still debatable as there is a lack of sufficient data. With the available information LR is the best treatment option in the case the patients liver function and performance status permits such approach. Moreover, the location of the tumor will also be part of the algorithm when making a decision on resecting the tumor. Ablative therapies such as RFA are an excellent alternative with good outcomes in case of very early HCC. The main counterpart to these treatments is the high rate of tumor recurrence. In this scenario (recurrence after primary treatment) LT can play an important role in the treatment of very early HCC. With the current allocation systems, patients with these tumors don't get exception

points and another interesting approach would be to wait and not treat until the tumor grows to get exception points. Further investigations on the best management of cirrhotic patients diagnosed of very early HCC are needed.

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TOPIC HIGHLIGHT

WJH 6th Anniversary Special Issues (2): Hepatocellular carcinoma

Mammalian target of rapamycin inhibition in hepatocellular carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is one of the leading causes of cancer-related death worldwide. It is associated with a poor prognosis and has limited treatment options. Sorafenib, a multi-targeted kinase inhibitor, is the only available systemic agent for treatment of HCC that improves overall survival for patients with advanced stage disease; unfortunately, an effective second-line agent for the treatment of progressive or sorafenib-resistant HCC has yet to be identified. This review focuses on components of the mammalian target of rapamycin (mTOR) pathway, its role in HCC pathogenesis, and dual mTOR inhibition as a therapeutic option with potential efficacy in advanced HCC. There are several important upstream and downstream signals in the mTOR pathway, and alternative tumor-promoting pathways are known to exist beyond mTORC1 inhibition in HCC. This review analyzes the relationships of the upstream and downstream regulators of mTORC1 and mTORC2 signaling; it also provides a comprehensive global picture of the interaction between mTORC1 and mTORC2 which demonstrates the pre-clinical relevance of the mTOR pathway in HCC pathogenesis and progression. Finally, it provides scientific rationale for dual mTORC1 and mTORC2 inhibition in the treatment of HCC. Clinical trials utilizing mTORC1 inhibitors and dual mTOR inhibitors in HCC are discussed as well. The mTOR pathway is comprised of two main components, mTORC1 and mTORC2; each has a unique role in the pathogenesis and progression of HCC. In phase Ⅲ studies, mTORC1 inhibitors demonstrate anti-tumor activity in advanced HCC, but dual mTOR (mTORC1 and mTORC2) inhibition has greater therapeutic potential in HCC treatment which warrants further clinical investigation.

Key words: Mammalian target of rapamycin; hepatocellular carcinoma; Mammalian target of rapamycin complex 1; Mammalian target of rapamycin complex 2; PI3K/AKT/mTOR signaling pathway; Sorafenib; Everolimus; Sirolimus; Liver transplantation; CC-223

Core tip: Advanced hepatocellular carcinoma (HCC) has a poor prognosis with limited therapeutic options. The mammalian target of rapamycin (mTOR) pathway (regulated by mTORC1 and mTORC2) is implicated in HCC pathogenesis. This review examines pre-clinical and clinical data demonstrating that mTORC1 inhibition effectively prevents HCC recurrence post-liver transplantation, and also has a modest anti-tumor effect in advanced HCC. The rationale and preclinical data for utilizing dual mTOR (mTORC1 and mTORC2) inhibition in HCC is also reviewed; a current phase Ⅰ clinical trial to investigate the efficacy of dual mTOR inhibitors is briefly discussed. mTOR pathway inhibition has therapeutic potential in the treatment of advanced HCC.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer diagnosed world-wide, with 250000 to 500000 cases diagnosed per year, and it is the third leading cause of cancer-related death in the world[1]. In the United States, the incidence of HCC has nearly tripled over the past 3 decades, with approximately 20000 new cases diagnosed annually, largely owing to the growing incidence of chronic Hepatitis C-related cirrhosis. HCC is associated with a poor prognosis, with 5-year survival rate persistently less than 10%. It is potentially curable by surgery or liver transplantation if detected early. Unfortunately, over 85% of cases are diagnosed at late stages when surgical intervention is no longer a viable option. The only available systemic treatment is a multi-targeted kinase inhibitor, sorafenib. In randomized, placebo-controlled phase Ⅲ clinical trials, sorafenib modestly improves overall survival (OS) for patients with intermediate to advanced stage HCC[2,3]. An effective second-line agent for those with sorafenib failure or intolerance has yet to be identified. This has led to an ongoing search for molecular pathways and novel compounds for the treatment of advanced HCC.

Mammalian target of rapamycin (mTOR) is a serine/threonine protein kinase downstream of the phophoinositide-3-kinase (PI3K)-related kinase family. It is a central regulator of various oncogenic processes including cell growth, proliferation, metabolism, and angiogenesis. There is growing evidence to suggest that mTOR deregulation plays a significant role in hepatocellular carcinogenesis. Pre-clinical data indicates that deregulated expression of mTOR pathway effectors is present in 40%-50% of HCCs, and activation of the mTOR pathway is associated with less differentiated tumors, earlier tumor recurrence, and worse survival outcomes[4]. Our review focuses on components and functions of the mTOR pathway and its potential role in the treatment of advanced HCC.

MTOR PATHWAY

Components of mammalian target of rapamycin complexes

The PI3K/AKT/mTOR signaling pathway- also known as the "mTOR pathway"- contains two important components: mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) (Figure 1). They are multiprotein complexes, comprised of both shared and unique components.

The mTOR kinase- also known as "mTOR"- is one of three components which is present in both mTORC1 and mTOR2; mammalian lethal with SEC13 protein 9 (mLST8) and DEP domain-containing mTOR-interacting protein (DEPTOR) are two other proteins that are common to both mTORC1 and mTORC2. mLST8 interacts directly with mTOR to enhance its kinase activity, particularly within mTORC2 (its effect within mTORC1 is not clearly understood)[5]. DEPTOR prevents substrate binding to mTORC1 and mTORC2, which leads to inhibition of mTORC1 and mTORC2 activity[6,7].

mTORC1, which is sensitive to the effects of rapamycin, has two unique proteins: regulatory-associated protein of mTOR (RAPTOR) and 40 kDa Pro-rich AKT substrate (PRAS40; also known as AKT1S1). RAPTOR serves as a binding platform where substrates are presented to mTOR for subsequent activation of mTORC1[8]. Conversely, PRAS40, like DEPTOR, is a direct inhibitor of mTORC1 substrate binding which hinders mTORC1 activity[9].

Specific to mTORC2 are rapamycin-insensitive companion of mTOR (RICTOR), mammalian stress-activated map kinase-interacting protein 1 (mSIN1; also known as MAPKAP1) and protein observed with RICTOR (PROTOR) (Figure 1)[10]. There is some evidence that RICTOR contributes to the structural foundation of mTORC2; in the absence of RICTOR, mTORC2 becomes inactive[7]. The functions of mSIN1 and PROTOR remain unclear.

Functions and regulations of mTORC1 and mTORC2

mTORC1: mTORC1 expression is driven by stimulants such as energy status, physiologic stress, and growth factors. Specifically, in the presence of growth factors, insulin receptor substrate 1 (IRS1) activates PI3K. PI3K phosphorylates the second messenger called phosphatidylinositol (4,5)-biphosphate (PIP-2), which becomes phosphatidylinositol (3,4,5)-triphosphate (PIP-3) upon phosphorylation. PIP-3 then promotes the phosphorylation of serine/threonine protein kinase (PKB/AKT) at protein residue Thr308 by 3-phosphoinositide-dependent protein kinase-1 (PDK1). Further downstream signaling through the effector tuberous sclerosis 1-tuberous sclerosis 2 complex (TSC1-TSC2) ultimately leads to the activation of mTORC1.

Activated mTORC1 phosphorylates its two downstream targets, 70S ribosomal protein S6 kinase (S6K1) and the eukaryotic initiation factor 4E binding protein 1 (4E-BP1). S6K1 and 4E-BP1 are major regulators of protein translation; they also drive cell proliferation, angiogenesis, and autophagy[11].

Under normal physiologic conditions, 4E-BP1 binds to the eukaryotic initiation factor 4E (eIF4E) to arrest protein translation. However, when 4E-BP1 is phosphorylated by mTORC1, its binding to eIF4E is interrupted, and this allows protein translation to occur. Concomitantly, phosphorylation of S6K1 by mTORC1 also results in protein translation; however, it also creates a negative feedback loop whereby the phosphorylated S6K1 attenuates PI3K signaling by suppressing IRS1 activity, leading to mTORC1 inhibition. Interestingly, mTORC1 inhibition by rapamycin and its analogues disrupts S6K1-mediated feedback inhibition of PI3K signaling, which allows for increased PKB/AKT phosphorylation (Figure 2).

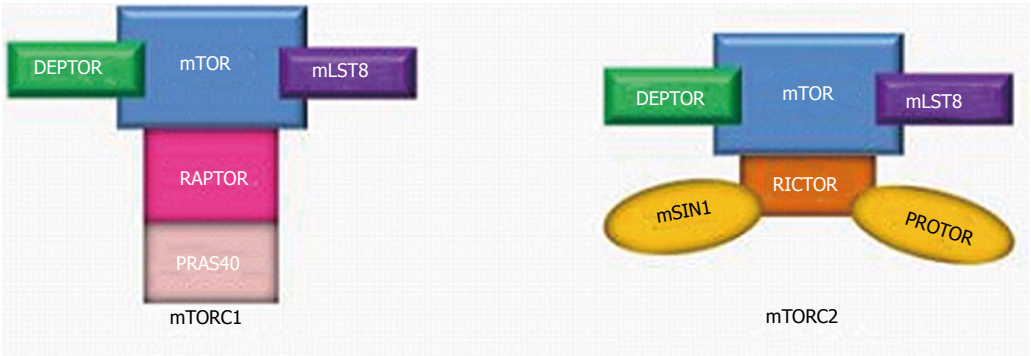

Figure 1 Mammalian target of rapamycin complex 1, Mammalian target of rapamycin complex 2 and their associated proteins. mTOR: Mammalian target of rapamycin; DEPTOR: DEP domain-containing mTOR-interacting protein; mLST8: Mammalian lethal with SEC13 protein 9; RAPTOR: Regulatory-associated protein of mTOR; PRAS40: 40 kDa Pro-rich AKT substrate; RICTOR: Rapamycin-insensitive companion of mTOR; mSIN1: Mammalian stress-activated map kinase-interacting protein 1; PROTOR: Protein observed with RICTOR.

Furthermore, rapamycin-induced inhibition of mTORC1 leads to an accumulation of phosphorylated AKT which can then activate downstream effectors of alternative pathways to inhibit apoptosis and promote cell proliferation[12].

mTORC2: Similar to mTORC1, mTORC2 activity is also promoted by growth factors. The upstream regulatory mechanisms specific to mTORC2 are poorly understood; however, its role in the phosphorylation of PKB/AKT has been well-characterized. The full activation of PKB/AKT requires two steps of phosphorylation: first, at protein residue Thr308 by PDK1, and second, at residue Ser473 by mTORC2[13]. Therefore, mTORC2 indirectly promotes mTORC1 activity through activation of PKB/AKT (Figure 2). Another less understood function of mTORC2 involves the regulation of actin cytoskeleton organization. Unlike the inhibitory effects on mTORC1, the effects of rapamycin and its analogues on mTORC2 are minimal[14].

Constitutive upstream regulators of the mTOR pathway: PTEN and TSC1-TSC2 complex

Phosphatase and tensin homologue on chromosome 10 gene (*PTEN*) is a multiphosphatase tumor suppressor located on human chromosome 10q23.3. It blocks the downstream activity of PI3K-AKT signaling by degrading PIP-3[11]. Inhibition of PIP-3 by PTEN prevents activation of PKB/AKT which leads to the down-regulation of mTORC1 activity (Figure 2). In the absence of PTEN, activation of mTORC1 is unbridled and hepatocellular carcinogenesis occurs. Watanabe *et al*[15] showed high incidence of HCC (66%) in PTEN-deficient mice at the end of an 80-wk period. Wang *et al*[16] demonstrated that decreased PTEN protein expression in HCC tissue samples compared to paired surrounding tissue samples was associated with higher tumor pathologic grade, TNM stage, and more frequent incidence of metastasis.

The TSC1 and TSC2 proteins (also known as hamartin and tuberin, respectively) are regulators of cell proliferation which have been implicated in HCC carcinogenesis. In its active form, the TSC1-TSC2 complex inhibits mTORC1 activation. Specifically, TSC2 acts as a GTPase-activating protein (GAP) which degrades guanosine triphosphate (GTP) and prevents its binding with Rheb, a GTP-binding protein. As a result, Rheb's ability to inhibit FKBP38, a negative regular mTORC1, is disabled and mTORC1 is inhibited. However, Akt-mediated phosphorylation deactivates the TSC1-TSC2 complex by decreasing its GAP-activity towards Rheb, which permits Rheb-GTP binding[17]. In turn, Rheb carries out its usual function of inhibiting FKBP38, and mTORC1 activation occurs (Figure 2)[17,18]. In fact, loss of either TSC1 or TSC2 promotes autonomous activation of mTORC1. Using liver-specific TSC1 knockout mice, Menon *et al*[19] demonstrated that chronic activation of mTORC1 in the absence of TSC1 induced hepatocyte damage, independent of hepatic steatosis, which leads to the spontaneous development of HCC.

It is important to note that PTEN and TSC1-TSC2 complex also function as integrating hubs for the regulation of mTOR *via* alternative signaling pathways. For example, the Src family kinases (SFKs) and the Wnt protein of the Wnt/β-catenin pathway are direct upstream regulators of PTEN and TSC1-TSC2 complex, respectively. Studies of breast cancer cell lines have shown that SFKs phosphorylate PTEN to inhibit its function[20], which then promotes mTORC1 activation. Conversely, the stimulation of Wnt prevents TSC2 phosphorylation through inhibition of GSKβ3, a protein constituent of Wnt/β-catenin pathway, thus inhibiting mTORC1 activation[17].

CLINICAL EXPERIENCE OF MTOR INHIBITION IN HCC

mTORC1 inhibitor in the prevention of HCC recurrence post liver transplantation

Within the past decade, the role of mTOR inhibition in the prevention of HCC recurrence has been examined more thoroughly in the post-liver transplantation patient population. Recurrence is a major cause of morbidity and mortality among these patients, and the recurrence risk is markedly influenced by explant pathology such as poor tumor differentiation and the presence of microvascular invasion[21].

The traditional immunosuppressants used to prevent

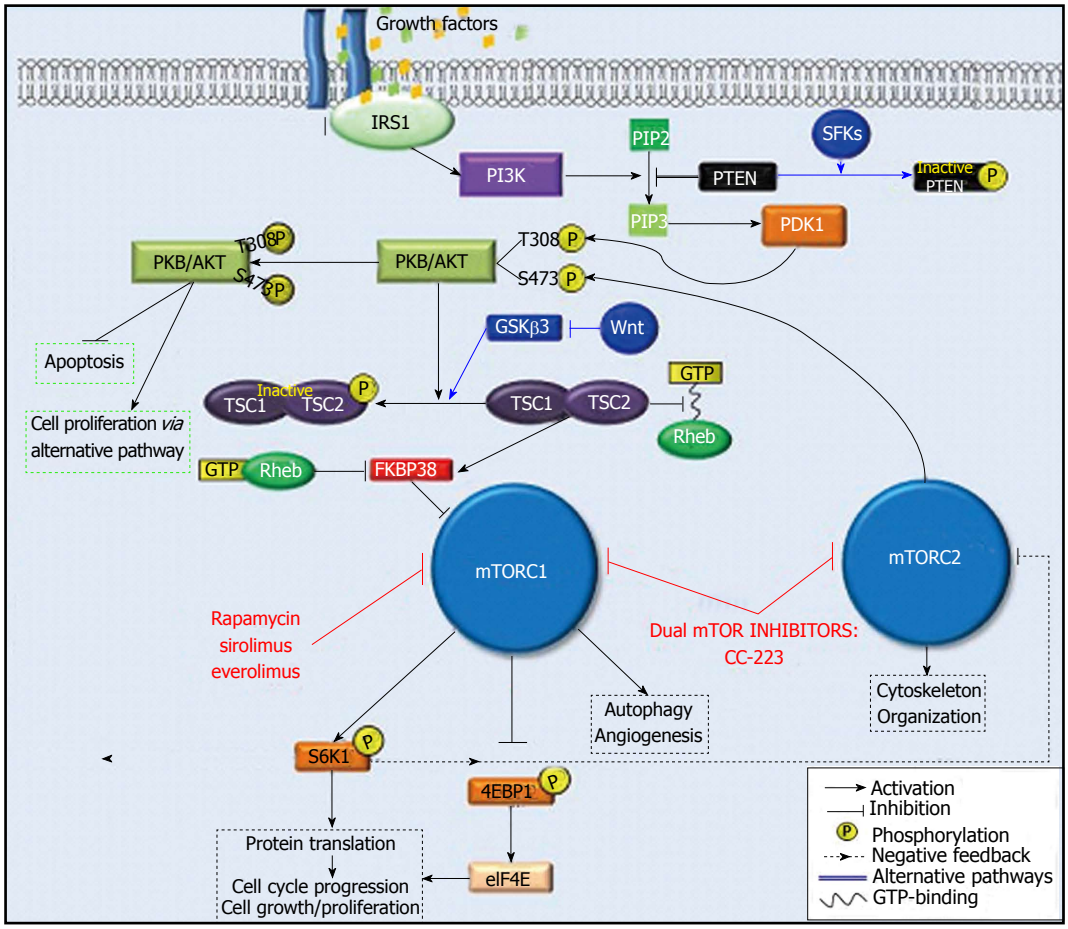

Figure 2 The PI3K/AKT/mTOR pathway. IRS1: Insulin receptor subsrate 1; PI3K: Phophoinositide-3-kinase; PIP-2: Phosphatidylinositol (4,5)-biphosphate; PIP-3: Phosphatidylinositol (3,4,5)-triphosphate; PTEN: Phosphatase and tensin homologue on chromosome 10 gene; PDK1: Phosphoinositide-dependent protein kinase-1; PKB/AKT: Serine/threonine protein kinase; TSC1-TSC2: Tuberous sclerosis 1-tuberous sclerosis 2 complex; 4EBP1: Eukaryotic initiation factor 4E binding protein 1; eIF4E: Eukaryotic initiation factor 4E; S6K1: 70S ribosomal protein S6 kinase; SFKs: SRC family kinases.

liver allograft rejection are calcineurin inhibitors (CNIs) such as tacrolimus and cyclosporine. They have been implicated in tumorogenesis both *in vitro* and *in vivo*[22,23]. In contrast, mTOR inhibitors are capable of effective immunosuppression (by blocking interleukin-2-mediated acute graft rejection) and concomitant prevention of hepatocellular tumorogenesis (through potent inhibition of angiogenesis). These two reasons make them attractive immunosuppresants for post-liver transplantation patients with a pre-transplant diagnosis of HCC[24].

In retrospective and non-randomized prospective analyses, post-liver transplantation HCC patients treated with sirolimus (a rapamycin analogue which selectively inhibits mTORC1) showed decrease in HCC recurrences[25]. In a study of 70 post-liver transplantation HCC patients treated with sirolimus-based immunosuppression, Toso *et al*[26] demonstrated an absolute decrease in recurrence rates by 6% (Milan criteria) and 14% (beyond Milan criteria) compared to studies not using sirolimus. A recent meta-analysis in patients with HCC who underwent liver transplantation indicated that sirolimus-treated patients demonstrated longer 5-year relapse-free survival (RFS) and 5-year OS rates (79%-80% and 80%, respectively) compared to CNI-treated patients (54%-60%; 59%-62%, respectively)[27].

Because of this promising data, a prospective, randomized international clinical trial (the "SiLVER trial") has been developed to assess the role of sirolimus in HCC-free patient survival in liver transplantation recipients with a pre-transplant diagnosis of HCC; the primary endpoint is RFS with a planned 5-year follow-up[28].

mTORC1 inhibitor in advanced HCC

Recently, single-arm phase Ⅰ/Ⅱ studies have shown that everolimus (a second-generation mTORC1 inhibitor), has single-agent activity in de novo or recurrent advanced HCC. In a cohort of 36 patients, everolimus hindered disease progression in patients with advanced HCC when used at maximum tolerated dose of 70 mg weekly[29]. In a subsequent phase Ⅰ/Ⅱ study by Zhu *et al*[30], 28 patients with advanced HCC tolerated everolimus at the dose of 10 mg daily. The median progression free survival was 3.8 mo, suggesting a modest antitumor effect of everolimus in advanced HCC[31].

This study led to the global phase Ⅲ randomized EVOLVE-1 trial, where everolimus was compared to placebo in patients with advanced HCC who discontinued sorafenib due to disease progression or drug intolerance. This trial unfortunately showed no OS benefit for

everolimus in the salvage setting of advanced HCC[32]. As discussed in section 2 (b) of this review, mTORC1 and mTORC2 are two complementary components of the mTOR pathway: when mTORC1 is inhibited, mTORC2 is upregulated. This increase in mTORC2 activity generates a surplus of phosphorylated PKB/AKT which, despite mTORC1 inhibition, inhibits apoptosis and promotes cell proliferation *via* alternative pathways (Figure 2)[33]. This phenomenon may partially explain the unsatisfactory efficacy of everolimus demonstrated in the EVOLVE-1 trial, and suggests a potential mechanism for drug resistance against mTORC1 inhibitors in HCC. Given this theory, dual mTORC1 and mTORC2 inhibition has become an attractive pharmacologic target with therapeutic potential in advanced HCC treatment.

The safety of everolimus in combination with sorafenib has also been evaluated for the treatment of advanced HCC, as it posed the opportunity to target two major pathways involved in HCC pathogenesis. However, phase I studies demonstrated intolerable toxicities with this combination, rendering it infeasible as a therapeutic option[34,35].

POTENTIAL OF DUAL MTOR INHIBITION IN HCC

Pre-clinical studies using second generation mTOR inhibitors (*i.e.*, Pp242, OSI027, AZD8055) in HCC cell lines and xenograft models have demonstrated enhanced antitumor efficacy of dual mTORC1/2 targeting[36-38]. Specifically, CC-223 (CC0482223) is a potent selective inhibitor of both mTORC1 and mTORC2 that impedes tumor resistance by inhibiting AKT phosphorylation. In multiple tumor cell lines, substrates of both mTORC1 and mTORC2 (p-S6RP and pAKT Ser473, respectively) were inhibited by CC-223, whereas rapamycin was a successful inhibitor of its downstream target p-S6RP only.

The therapeutic potential of CC-223 is being tested in a phase I trial of patients with refractory malignancies including HCC. Twenty-seven HCC patients have been enrolled as of June 2013; 93% of them previously received sorafenib. With 45 mg daily dosing of CC-223, 11% of patients exhibited a partial response, and 33% of patients maintained stable disease[39]. Due to this encouraging data, a cohort expansion of CC-223 in HCC patients is ongoing.

FUTURE DIRECTIONS IN MTOR INHIBITION FOR HCC

HCC undergoes constant mutational changes throughout its carcinogenesis and progression; therefore, combination therapy may be of interest. The possibility of non-overlapping pathway inhibition can be considered. For instance, sorafenib and dual mTOR inhibition could be a potentially effective strategy. In addition, epigenetic modification through methylation contributes to therapy resistance in many tumor types and HCC is no exception[40]. Dual mTOR inhibition combined with demethylating agents could also be a valid scientific approach[41].

Dramatic advances in the treatment of HCC have been achieved with improvement in the understanding of the biology of HCC pathogenesis and progression. The mTOR pathway is clearly critical to the progression of HCC. We anticipate that future data on single-agent dual mTOR inhibitors and combination strategies utilizing mTORC dual inhibition with other novel agents will contribute to the advances in HCC treatment.

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WJH 6th Anniversary Special Issues (2): Hepatocellular carcinoma

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Abstract

The incidence of hepatocellular carcinoma (HCC) is known to be high in West Africa with an approximate yearly mortality rate of 200000. Several factors are responsible for this. Early acquisition of risk factors; with vertical or horizontal transmission of hepatitis B (HBV), environmental food contaminants (aflatoxins), poor management of predisposing risk factors and poorly-managed strategies for health delivery. There has been a low uptake of childhood immunisation for hepatitis B in many West African countries. Owing to late presentations, most sufferers of HCC die within weeks of their diagnosis. Highlighted reasons for the specific disease pattern of HCC in West Africa include: (1) high rate of risk factors; (2) failure to identify at risk populations; (3) lack of effective treatment; and (4) scarce resources for timely diagnosis. This is contrasted to the developed world, which generally has sufficient resources to detect cases early for curative treatment. Provision of palliative care for HCC patients is limited by availability and affordability of potent analgesics. Regional efforts, as well as collaborative networking activities hold promise that could change the epidemiology of HCC in West Africa.

Key words: Liver cancer; West Africa; Aflatoxin; Surveillance; Hepatitis B

Core tip: It is known that outside the region of East Asia, sub-Saharan Africa has the highest incidence of hepatocellular carcinoma (HCC). Within Africa the West African region remains the focus of significant disease activity. We reviewed the main issues responsible for this pattern. Although intervention efforts, such as primary prevention through hepatitis B vaccination, has led to reductions of chronic hepatitis B infection in some countries such as Gambia and Senegal, there remains a huge gap in secondary prevention, which are responsible for continuing high mortality to incidence ratio of HCC in West Africa. Collaborative clinical care and basic science translational research holds promise towards changing the current trend.

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INTRODUCTION

Hepatocellular carcinoma (HCC) constitutes almost 85% of all primary liver cancers, and is known to be the 5th most commonly diagnosed cancer globally[1]. In 2012, about 782000 and 746000 new cases and deaths respectively, had HCC as their primary diagnosis[2]. Sub-Saharan Africa is the most affected region of the world, after Eastern Asia owing to the high prevalence of risk factors for this cancer in these continents. Although a description of the burden of HCC in developing countries, highlighting the sub-Saharan African situation has recently been reported by Kew[3], the countries of West Africa have reported more than average incidence of HCC, a situation deserving in depth understanding. In this article, we systematically reviewed the problem of HCC in West Africa: contributing factors, primary and secondary prevention efforts, as well as the provision of palliative care to patients. This review provides an overview of current efforts and suggests opportunities for strategic intervention.

EPIDEMIOLOGY OF HCC IN WEST AFRICA

There is a high incidence of HCC in West Africa. In countries like Gambia, Guinea-Conakry and Sénégal, the incidence of HCC has been reported to range between 30-50/100000 and 12-20/100000 in men and women, respectively[4]. The West African region comprises 16 countries. It has an area approximating 6.1 million km^2, bordered in the north by the Sahara desert and the east by Mount Cameroon and Lake Chad. Aside from shared economic interests, such as the Economic Community of West African States, there are similarities in the dress, cuisine, music and culture of people living in this geographical enclave. These factors may indeed underlie the way that HCC presents.

The mortality rate of HCC is almost the same as its incidence in this region of the world. Individual national cancer registry data are limited. However, the global cancer registry database has provided estimates of incidence and mortality by gender for primary liver cancer; of which HCC constitutes approximately 85%. Taking into account the incompleteness of cancer registration in this region, these data highlight the high case fatality rate of HCC. The most affected country is The Gambia, followed by Guinea, Liberia and Sierra-Leone in that order (Figure 1).

As most affected persons are middle-aged, HCC contributes to decreased economic development of this region. Whereas the incidence of HCC in most developed countries show that the highest affected is 75 years and older, and similar patterns among high risk Asian populations, the situation is different in West Africa. There is a significant male preponderance of this tumour, being the most commonly encountered malignancy in men in several West African countries (Table 1). The rates of HCC in men in countries like Gambia and Mali tend to peak at 60 to 65 years while females peak between 65 and 75 years[5]. A study has reported peak age of 40 years from this region[6].

Some reasons for the characteristic epidemiological pattern of HCC in West Africa are discussed as follows.

Failure to identify at risk populations

It is not uncommon for some patients in West Africa to be found to have hepatitis B viral infection, for the first time, when they present to hospitals with decompensated liver disease. This late diagnosis is not only as result of lack of health-seeking behaviour, but likely to be due to some additional factors. As the performance of healthcare delivery is often suboptimal in this region, many hepatitis B surface antigen (HBsAg)-positive patients seek herbal and alternative medications as the initial port of call prior to attending orthodox care. Since few individuals receive adequate management for chronic hepatitis B, there is a tendency to progress to cirrhosis. Malignancy, on the background of poor hepatic reserve, with additional consumption of traditional remedies; of unknown toxicities, tip the patients to liver failure on first hospital presentation.

Low hepatitis B virus immunisation adherence

Significant declines in HBsAg prevalence and low rates of childhood HCCs have been realised in countries that introduced Hepatitis B virus (HBV) vaccine[7]. One study in the region has revealed that HBV vaccination is capable of decreasing chronic HBsAg carriage by up to 83%[8]. This observation has been replicated in studies from Senegal and South Africa[9,10]. However, many countries in the region have ensured complete adherence to whole course of HBV vaccination. The Global Alliance for Vaccines and Immunisation funding and the World Health Organisation (WHO), supporting HBV vaccination programmes, have played an important role in the implementation of HBV vaccine programmes in Africa. Despite this effort, HBV vaccine coverage remains low estimated at 70% according to the WHO/UNICEF 2012 data.

Poor treatment of liver diseases

The treatment of liver diseases is generally inadequate in many countries of West Africa. Large number of patients overwhelms the limited number of trained medical personnel, inadequate infrastructure for training curricula, mass emigration of medical professionals and paucity of clinical guidelines adapted to the local setting are important in this regard. It was not until recently that hepatitis C virus (HCV) treatment guidelines for low and middle

Table 1 Summary of some studies indicating male preponderance of hepatocellular carcinoma relative to other cancers in West Africa

Country	Liver cancer relative to cancer elsewhere	Source of study population	Coverage
Niger[55]	Most common in men; M:F ratio of 1.4:1	National cancer registry	National
The Gambia[56]	Most common in men; 2nd in women	National cancer registry	National
Ghana[57]	Most common in men, 3rd in women; M:F ratio of 1.2:1	Southern Ghana	Mortality data from a tertiary centre
Nigeria[58]	Most common in men; M:F ratio of 2.4:1	South East Nigeria	Cancer mortality data
Nigeria[59]	Most common in 50-59 years old	South West Nigeria	Pathology reports
Côte d'Ivoire[60]	Second in men; less common in women	Cancer registry	National
Mali[61]	Most common cancer in men; cervical cancer leads in women	Cancer registry	National
Guinea-Conakry[62]	Most common in men; second in women	Cancer registry	Regional

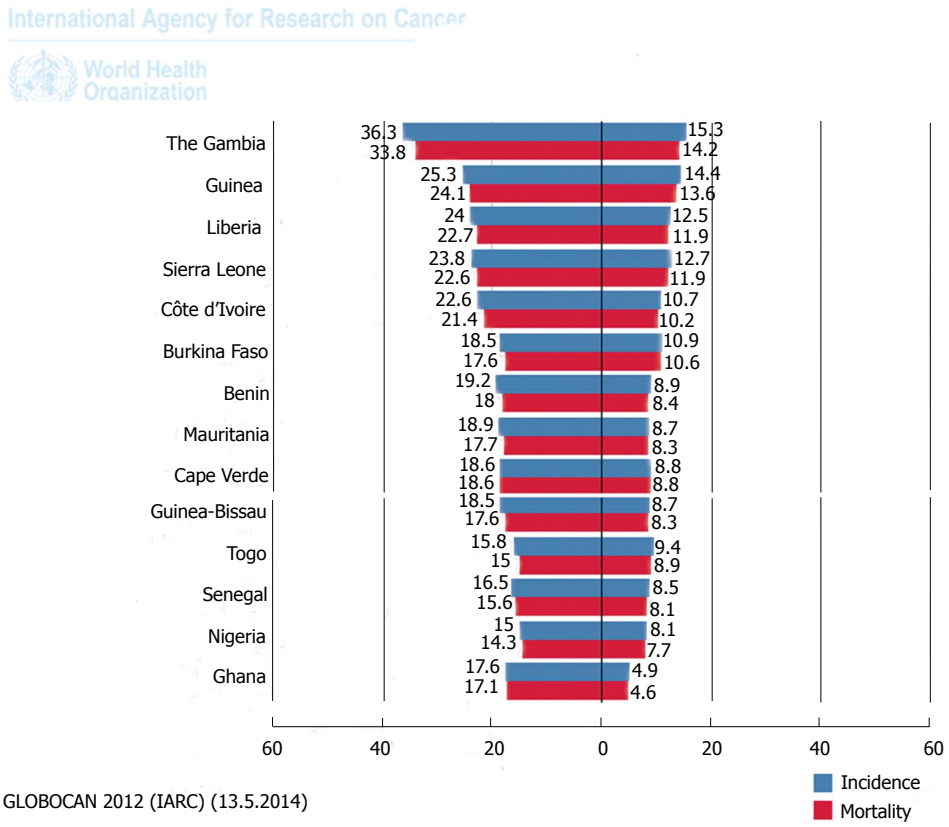

Figure 1 Multiple clustered bar charts labelled by incidence and mortality rates per 100000 population of West African countries (data from Globocan 2012 from International Agency for Research on Cancer[54].

income countries were commissioned by the WHO[11]. Inadequate funding prevents the optimal treatment of those affected, as the cost of these medications is prohibitive for most sufferers in these countries[12]. Patients tend to present to hospitals when they have noticed symptoms or when a close relative gets diagnosed with an associated complication of viral hepatitis.

Inadequate public health intervention

The burden of disease imposed by viral hepatitis has been completely ignored by the international health agenda these last decades as the focus has been put on human immunodeficiency syndrome (HIV), tuberculosis, and malaria, three major infectious diseases issues which have been the main recipient of health care resources and funding[13]. Yet, if the mortality and morbidity from cirrhosis and liver cancer were grouped, the burden of viral hepatitis would have to be seriously considered by the international health authorities[14]. The lack of public health campaigns is complicated by a plethora of traditional healers.

There is also a scarcity of coordinated health programmes that could inform governments in the region regarding the problem of liver diseases. With significantly high prevalence of HBsAg and anti-HCV in Nigeria, it was only in 2009 that a guideline for the treatment of HBV was produced. Similarly, the WHO and World Hepatitis Alliance estimate that only 17 countries in the whole of Africa that have designed national guidelines on viral hepatitis, of which only 3 sub-Saharan African countries (Cameroon, Rwanda and Mauritania) have implemented guidelines on HBV mother-to-child transmission. With

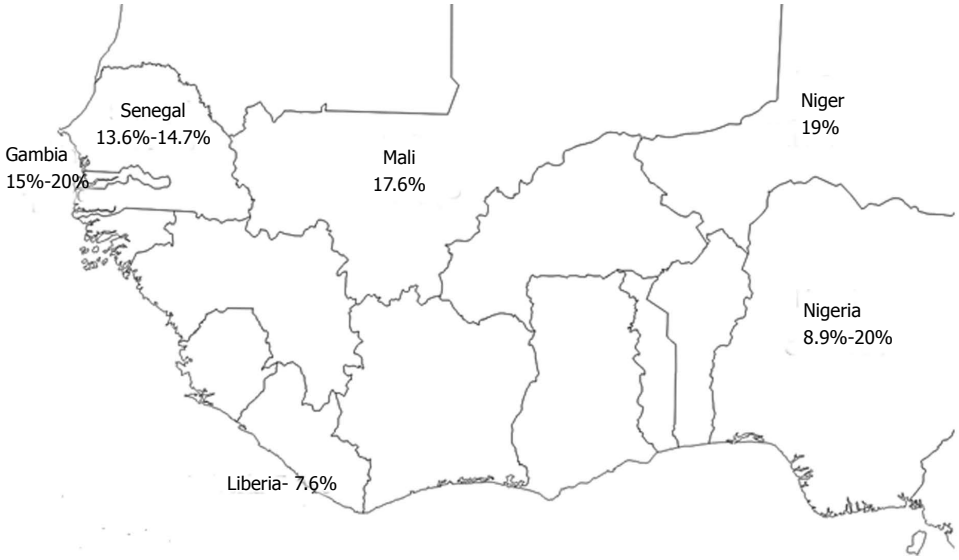

Figure 2 Prevalence of hepatitis B surface antigen carriage in some West African countries.

the prohibitive cost of antivirals and a fee-for-service system of healthcare, only those who can afford medication get treated. This ultimately results in a large pool of those who could have been treated for viral hepatitis going on to develop HCC.

HIGH RATE OF RISK FACTORS

Hepatitis

HBV, described as a potent carcinogen, is endemic in West Africa demonstrating varied prevalence. The infection rates of HBV vary between 8%-20% in this region[15].

West Africans have longer durations of HBV infections relative to individuals in the developed world who tend to get the infection much later in life. By age of 10 years, 80% of infected people in Africa have acquired HBV[16] and a high carriage rate of up to 20% (Figure 2). Inadequate data in the literature from this region may actually be modulating the true problem of HBV and its sequelae in the West African region.

HCV infection affects more than 8.4 million people in West Africa[11]. Although the transmission route of HCV in this region is not well established, and most cases are thought to be due to use of unsterile sharps, and receipt of unscreened blood products, sexual transmission may have a modest effect[17]. Owing to incorrect assumptions, anti-HCV serology was not part of routine screening for blood products until early 2000s. However, it is now known that the prevalence of HCV in West Africa varies from 2.5%-9.9%[17]. Although less prominent than HBV, HCV contributes to HCC in West Africa, particularly for those above 60 years[5].

The distribution of HBV genotype differs from one region of the world to another, and which could be a determinant factor in the clinical outcome of HBV infection. For example, in central and West Africa genotype E has been documented to predominate[18,19]. Studies from Asia have demonstrated an increase in the development of HCC among patients with HBV genotype C, compared to genotype B[20-23]. A study in South Africa has shown that HBV genotype A had a greater hepatocarcinogenic potential than other non-A genotypes[24].

Aflatoxin

This mycotoxin, produced by the fungus *Aspergillus spp*., grows on mainly legumes and cereals in humid conditions in parts of West Africa. These foods are mostly consumed unprocessed as staples in West Africa. Subsistence farming, poor farm produce storage and suboptimal processing systems facilitate widespread exposure tothis toxin (Figure 3). Aflatoxin and HBV infection can synergistically increase the risk of HCC. A possible molecular mechanism has been suggested by studies in HBV transgenic mice[25]. That study suggested that chronic inflammatory damage of the liver alters the expression of carcinogen metabolizing proteins and may thus moderate the binding of aflatoxin to DNA. Further research in the region has confirmed a significant increase in the risk of cirrhosis in patients exposed to aflatoxin[26]. Additional research in this area could be far-reaching; and may enhance policy decisions regarding drying, storage, processing and consumption of foods such as cereals that are consumed in large amounts in the countries within this region.

Alcohol

Although not as affluent as developed countries, alcohol consumption goes on, albeit to an undocumented level in West Africa. Locally-brewed fermented drinks in Africa have been reported to significantly contribute to HCC. These studies postulated that the brewing containers (Figure 4) release iron in consumers of these drinks, which leads to an iron overload syndrome. Almost a tenth of some populations in sub-Saharan Africa have been noted to have iron overload[27,28]. Iron levels have

Figure 3 Fungus-infested malt, a product of cereal used in the fermentation of local alcohol beverage "burukutu". Cereals are widely consumed in West Africa and are a source of aflatoxin, which has been shown to potentiate the hepatocarcinogenicity of hepatitis B virus (Picture by Pantong Mark at Jos, Nigeria).

Figure 4 Iron pots used in brewing local beer in a typical West African setting (Picture by Pantong Mark, Jos, Nigeria).

been reported to be significantly higher among Africans with liver cancer than controls[29]. A genetic polymorphism in the ferroportin-1 has been demonstrated in a southern African study population, and thought to be associated with decreased iron excretion[30]. The interaction between alcohol, HBV and iron overload could be far-reaching to predispose West Africans to HCC. Studies have found that the incidence of HCC is 200 fold in haemochromatosis if the patients are above 55 years of age, have HBsAg seropositivity and who additionally drink alcohol[31,32].

HIV and hepatitis co-infection

The impact of HIV infection on chronic viral hepatitis B and C, as well as on the response to hepatitis B immunisation antibody generation are subjects deserving further studies in this region. Data from developed countries have established definite links between HIV/HBV and HIV/HCV co-infections, as well as HCC[33,34]. Prior to the provision of highly active antiretroviral treatment to Africa, most HIV patients died earlier due to opportunistic infections before they developed complications of HBV or HCV. Recent experience emerging from well monitored HIV centres in West Africa[35] confirms that most co-infected patients are expected to survive longer and the impact on the overall burden of HCC will eventually emerge. Furthermore, the impact of HIV infection on the long-term efficacy of the HBV vaccine in West Africa remains to be determined and might pose serious consequences for the gains already made in places that have attained a wide HBV vaccination coverage[36].

CLINICAL PRESENTATIONS OF HCC IN WEST AFRICA

The natural history of untreated HCC and the associated clinical features have been well characterised from developed countries[37]. Early HCC is often asymptomatic and is devoid of pathognomonic features. Certain features that distinguish HCC presenting in developed countries relative to West African countries are summarised in Table 2. Whereas 5%-10% of HCC patients in the West and almost 30% in Japan are diagnosed with early disease[38], almost all persons diagnosed with HCC in West Africa are diagnosed very late[5,39]. The presence of a painful right upper abdominal mass and swelling, weight loss and early satiety signify advanced disease[40].

Weight loss is the commonest symptom of HCC, often attributed to "witchcraft" in West African populations. Right upper abdominal pain, abdominal swelling and jaundice are not uncommon. Other symptoms include anorexia and confusion. To ease diagnosis, most clinicians in sub-Saharan Africa recognise a prospective HCC patient either as: one with abdominal pain and a hard nodular hepatomegaly, or "a triad of abdominal pain, swelling and jaundice". A few studies from the region[5,41] have corroborated the stated features (Figure 5).

DIAGNOSTIC CHALLENGES OF HCC IN WEST AFRICA

Challenges of diagnosis of HCC in developing countries have been recently highlighted[3]. According to the international guidelines the diagnosis of HCC relies on specific radiological aspects using computed tomography (CT) or magnetic resonance imaging (MRI) scans and/or histopathological analysis. However, in sub-Saharan Africa, these diagnostic tools are rarely used in clinical practice because: (1) CT or MRI scans with contrast are not available in many countries or are expensive and not free of charge; and (2) liver biopsy is an invasive and costly procedure requiring well trained hepatologists, histopathologists and laboratory technicians, who are not always at post. Moreover most percutaneous liver biopsies are not image-guided and hence there is a high chance of mis-diagnosis. Owing to low sensitivity, serum alpha-fetoprotein (AFP) is no longer recommended by most international guidelines (indeed in some guidelines it is used in combination with radiological features) to be used for this purpose[42], although almost all centres in West Africa still rely on it. As one third of HCC do not secrete AFP,

Table 2 Relative differences in risk factors, clinical features, surveillance and management of hepatocellular carcinoma between West Africa and developed countries

Parameter	Developed countries	West African countries
Predominant risk factor	Hepatitis C virus[2,63]	Hepatitis B virus[5]
Predominant co-factor	Alcohol	Aflatoxin B1[64]
Peak incidence	8th decade[65]	5th decade[57]
Stage at presentation	High chance of early stage at diagnosis[38]	Often advanced stage at presentation[3]
Surveillance	Routine; although compliance is about 12% in a study in the United States[66]	Not known and not routine
Median survival	Overall survival of > 16 mo in a study from United States[67]	Most die at initial presentation
Diagnosis	Radiological (multi-phasic dynamic CT or MRI) ± liver biopsy[68]	Tumour markers (occasionally, grey-scale ultrasound scan ± liver biopsy)[12,48]
Treatment	Curative therapies and palliative care; according to guidelines	Mainly palliative; often suboptimal

CT: Computed tomography; MRI: Magnetic resonance imaging.

and most of the tertiary centres use only grey scale ultrasound scan systems, a lot of those patients with hepatic lesions are missed and/or confused with other common inflammatory conditions such as amoebiasis, peritoneal and hepatic tuberculosis, lymphoma, cholangiocarcinoma and secondary hepatic tumours. The import of the foregoing is the fact that the rates of HCC being reported are unlikely to reflect the true picture of the burden of the disease in West Africa.

TREATMENT OF HCC IN WEST AFRICA

Owing to very late presentations and poor health infrastructures, the outcome of HCC in West Africa is generally dismal and curative management is impossible, treatment only relying on palliative care for the most part. Yet, very few centres have proper palliative care, as opiates are often unavailable and healthcare workers are not trained to use them. The vast majority (80%-90%) of cancer patients in sub-Saharan Africa only seek medical attention when cancers have reached an advanced stage, where end-of-life strategies are the only option. In 2009, only 12% of cancer patients in sub-Saharan Africa with moderate to severe pain were estimated to have been treated with opioid analgesics, an essential component of palliative care[43].

The management of pain for palliative patients has been also hampered by lack of knowledge and training and apprehension that opioid analgesics would cause severe digestive side effects and addiction. The so-called "opiophobia", among healthcare professionals is frequently observed in Africa[44] and is known to lead to under-prescription of pain relief medication. In The Gambia, it was found that only 12 HCC patients (48%) of HCC patients receive analgesics without any oral morphine prescription (personal communication).

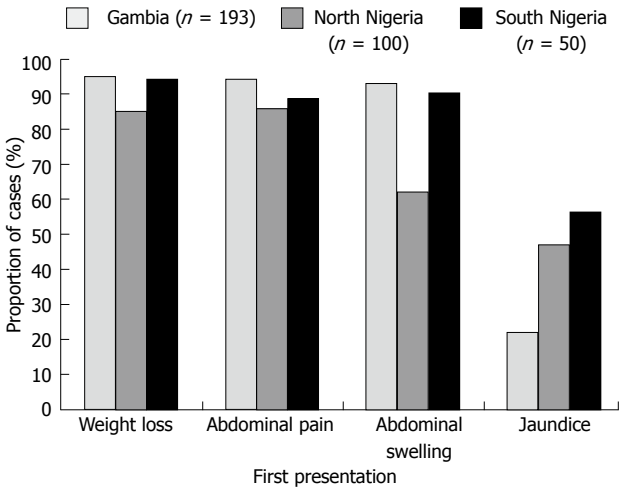

Figure 5 Summary of most common clinical presentations of hepatocellular carcinoma in three West African communities: Gambia, Northern and Southern Nigeria. Note the similarity of clinical presentation in three study sites in two countries in the region. Number of hepatocellular carcinoma patients in parentheses.

REASONS FOR LOW SURVIVAL OF HCC IN WEST AFRICA

Data on HCC survival in Sub-Saharan Africa are almost non-existent. A recent unpublished study conducted in Nigeria reported a median survival of 4 mo in 100 clinically diagnosed HCC patients[45] and preliminary data from the Prevention of Liver Fibrosis and Cancer in Africa programme in The Gambia found a median survival of 61 d (unpublished data). Worldwide data on cancer survival have shown that the 5-year relative survival was lowest in Uganda and Gambia, relative to other countries such as China[46].

Absent surveillance for HCC

In order to detect cases amenable to curative therapy, well-coordinated surveillance for HCC has to be in place. However, as this is not the case in most West African health centres, most HCC cases are detected at advanced stages[1]. Zhang *et al*[47] have recently reported the advantage of surveillance for HCC in at-risk populations in China, in which they noted a reduced mortality rate by 37% relative to a non-surveyed cohort[47]. For sub-Saharan Africa, serum AFP has been recommended for this purpose by the World Gastroenterology Organisation[48]. However, data on the adherence to this recommendation by physicians and compliance by patients are lacking. Data are available to support the fact that surveillance for HCC could improve therapeutic options for HCC[49].

Lack of treatment facilities for HCC

The advantage that surveillance provides would be confounded if treatment for HCC cannot be offered. Less complicated treatment modalities such as percutaneous ethanol injection of tumours could be offered only if the patients present at a relatively early stage. Liver transplant

services are scarce in West African countries. As fee-for-service continues to be the predominant health system in West Africa, specialists would not embark on skills that are rarely utilised.

Alternative treatment for "hepatitis"

There is a flourishing presence of self-acclaimed healers in West Africa (evangelical churches, as well as traditional religious practices) and claims of miraculous healing are an important contributor for the late presentation, as conventional western medical treatment is often a last resort for many of the afflicted.

CONCLUSION

Outlook and recommendations

HCC is a major cause of death in sub-Saharan Africa, estimated to be responsible for annual deaths of 200000 persons[50]. We have highlighted the direct and remote causes that may be contributing to the pattern of disease presentation in West African patients in this article. International and local efforts are underway to help regarding improving the current bleak outlook of this cancer. Deliberate attempts to reduce exposure to aflatoxin post-harvest had yielded encouraging results, which clinical significance could mean reduction of HCC development in at-risk persons[51]. Improvement of healthcare systems that could attract and retain specialists to tackle the risk factors and improvement in health budgetary implementation towards infrastructural facilities could provide a robust platform to changing the current trend.

In view of the multifactorial aetiological factors in the causation of HCC and the fact that little is in place regarding coordinated control of some of the these risk factors, some authors have predicted that the problem of HCC in West Africa is postulated to increase in the next 40-50 years[52]. However, this appears rather pessimistic and suggests that control efforts would not be in place. Already, some groups, such as the Prevention of Liver Fibrosis and Carcinoma in Africa (www.prolifica.eu) consortium have been investigating the impact of treatment of chronic HBV in reducing the incidence of HCC in this region. Already, this collaborative effort, comprising specialists from European and West African institutions, has led to identification of a validated panel of urinary metabolites[53] that could prove to be useful screening tool for HCC in West African populations in the future. Also, the activities of national professional bodies, such as the Society of Gastroenterology and Hepatology in Nigeria in publishing hepatitis treatment guidelines may only be effective if the West African community of states approach hepatitis in a logistical, programmed fashion, as has been done with HIV. More concerted attention is required to tackle HCC in West Africa in a comprehensive manner, involving public health personnel, hepatologists, oncologists, surgeons and palliative care practitioners.

We have thus presented a synopsis of how important HCC is in the West African region of the world; highlighting the high incidence, mortality and case fatality. Primary prevention methods such as HBV vaccination has led to reduction in chronic HBV infection, but its impact on reducing the incidence of HCC is yet to be documented in this sub region. Additionally, the contribution of aflatoxin deserves further study, as well as avoidance of its exposure aimed at maximising the prevention of liver cancer in this population should be a priority. There is hope in the horizon as there have been coordinated collaborative efforts to: (1) determine the impact of primary prevention in epidemiological terms; (2) provide primary prevention with programmes such as HBV vaccination (Gambia Hepatitis Intervention Study of the MRC); (3) secondary prevention and treatment of chronic HBV with the PROLIFICA programme; as well as (4) enhancing early detection of incident cases (PROLIFICA) in the region. Local efforts such as: provision of guidelines adaptable to the economic resources of the countries in the region as well as hepatitis awareness campaigns hold promise with assisting in the effort to curb this tumour. Parallel control efforts such as proper storage of cereals prior to consumption hold promise to reducing the synergistic contribution of aflatoxin to those already chronically infected by HBV. Results from these endeavours could potentially provide the platform to persuade governments in this region to facilitate larger scale universal policies.

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TOPIC HIGHLIGHT

WJH 6th Anniversary Special Issues (4): Cirrhosis

Role of vaptans in the management of hydroelectrolytic imbalance in liver cirrhosis

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Abstract

Ascites and hyponatremia are the most common complications in patients with liver cirrhosis and develop as a consequence of a severe impairment of liver function and portal hypertension. Increasing evidences support the central role of renal function alterations in the pathogenesis of hydroelectrolytic imbalances in cirrhotic patients, thus implying a dense cross-talk between liver and kidney in the systemic and splanchnic vascular homeostasis in such subjects. Since Arginin Vasopressin (AVP) hyperincretion occurs at late stage of cirrhosis and plays an important role in the development of refractory ascites, dilutional hyponatremia and finally hepato-renal syndrome, selective antagonists of AVP receptors V_2 (vaptans) have been recently introduced in the therapeutic algorithm of advanced cirrhotic patients. Despite the promising results of earlier phase-two studies, randomized controlled trials failed to find significant results in terms of efficacy of such drugs both in refractory ascites and hyponatremia. Moreover, concerns on their safety profile arise, due to the number of potentially severe side effects of vaptans in the clinical setting, such as hypernatremia, dehydration, renal impairment, and osmotic demyelination syndrome. More robust data from randomized controlled trials are needed in order to confirm the potential role of vaptans in the management of advanced cirrhotic patients.

Key words: Cirrhosis; Vaptans; Portal hypertension; Arginin vasopressin; Liver

Core tip: Increasing evidences support the central role of renal function alterations in the pathogenesis of hydroelectrolytic imbalances in cirrhotic patients. Since Arginin Vasopressin (AVP) plays an important role in the development of refractory ascites, dilutional hyponatremia and hepato-renal syndrome, selective antagonists of AVP receptors V_2 (vaptans) have been recently introduced in the therapeutic algorithm of advanced cirrhotic patients. Despite the promising results of earlier phase-two studies, randomized controlled trials failed to find significant results in terms of efficacy. Moreover, concerns on their safety profile arise. More robust data from randomized controlled trials are needed.

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INTRODUCTION

Ascites is the most common complication in patients with cirrhosis and develops as a consequence of a severe impairment of liver function and portal hypertension[1].

In fact, there is substantial evidence that severe portal hypertension is the main disorder in the occurrence of ascites in cirrhosis as ascitic patients have significantly higher portal pressure than those without ascites. In particular, an hepatic venous pressure gradient (estimation of the intrahepatic vascular resistance) of more than 12 mmHg has been found to cause the occurrence of ascites in cirrhotics[2,3].

Increasing evidences support the central role of renal function alterations in the pathogenesis of hydroelectrolytic imbalances in cirrhotic patients, thus implying a dense cross-talk between liver and kidney in the systemic and splanchnic vascular homeostasis in such subjects.

Each step of this cross-talk could represent a potential target for the pharmacological management of cirrhotic patients.

PATHOGENESIS OF ASCITES AND DILUTIONAL HYPONATREMIA

It is now evident that ascites is related more to alterations in the arterial vascular compartment and in kidneys than in the portal venous system[4].

Ascites has been traditionally considered as a consequence of backward transmission of the increased intrahepatic hydrostatic pressure into the splanchnic microcirculation and of the decrease in intravascular oncotic pressure because of the impaired hepatic synthesis of albumin.

More recently, the splanchnic arterial vasodilatation secondary to portal hypertension has been found as the central event of ascites formation in cirrhosis[5]. Such mechanism simultaneously induces two different types of events: a "forward" increase in capillary pressure because of a greater inflow of blood at high pressure into the splanchnic microcirculation with consequent passage of fluid into the peritoneal cavity, and the impairment of systemic hemodynamics and renal function, which leads to sodium and water retention[6].

Splanchnic and systemic vasodilatation is due to the excretion by sinusoids of vasoactive mediators, for instance nitric oxid (NO), together with glucagon and other vasodilator molecules[7]. As a consequence of this process, the efforts made by the organism in order to obviate to the imbalance in favor of vasoactive molecules generate a well described chain of processes leading to a "vicious" circle and hence an impairment of the above cited pathological events. Particularly, the followings steps are reported: (1) "underfilling" of effective arterial blood volume; (2) impairment of renal perfusion and deterioration of glomerular filtration rate; (3) release of catecholamines by sympathetic nervous system (SNS) as response to decreased volemia leading to increase in cardiac output and renal vasoconstriction; (4) hyperactivation of Renin-Angiotensin-Aldosterone system (RAAS) and secondary hyperaldosteronism; and (5) release of Adiuretin (ADH), also called arginin vasopressin (AVP), and decrease in levels of Atrial Natriuretic Peptide.

Initially, as long as cirrhosis is compensated and patients don't develop any hydro-electrolytic imbalance, the retained fluid volume suppresses renal reuptake of sodium and water and resets fluid balance, thus leading, together with the augmented cardiac output and cathecolamine incretion, to a general increase in arterial volemia. However, as the disease progresses the effective arterial blood volume isn't maintained any longer by the aforementioned mechanisms and ascites occurs as a consequence of the "vicious circle" due to the continuous retention of water and sodium by the kidneys[7,8].

Due to the above cited mechanisms, the decompensated cirrhotic patient, although the marked hydrosaline reuptake, presents hypovolemia, periferic arterial vasodilatation and tachycardia.

In the earlier phases of ascites onset, the RAAS and the SNS are not activated, hence the cause of sodium retention in this period is still unclear. Afterward, the two systems increase the release of mediators thus leading to further reduction in urine sodium excretion[7].

Finally, hyperincretion of ADH occurs and this explains the late onset of hyponatremia in decompensated cirrhotic patients. In fact, ADH is less sensitive than the SNS and RAAS to changes in the effective volemia[9].

Finally, in this setting, hepato-renal syndrome (HRS) occurs as extreme consequence of such imbalances (see below). The mechanisms leading to ascites occurrence are described in Figure 1.

RENAL ALTERATIONS IN CIRRHOSIS

As above specified, activation of RAAS has a key role in the occurrence of hydroelectrolytic imbalances in cirrhotics. Such activation, as well that of SNS and the hyperincretion of ADH, is a homeostatic response aimed at maintaining blood pressure at normal levels in cirrhotic patients with ascites. In fact, the infusion of selective antagonists of angiotensin II or antidiuretic hormone (V1 antagonists) to experimental animals or ascitic patients leads to a profound hypotensive response secondary to a decrease in peripheral vascular resistance[10]. Among the main stimuli leading to the activation of these systems, arterial vasodilatation and secondary arterial hypotension play a pivotal role.

Other system implied in the pathogenesis of renal sodium retention is the SNS, which increases sodium reabsorption in the proximal tubule, loop of Henle, and distal tubule[11]. As decompensated liver diseases progresses, patients develop a decreased renal ability to excrete free water. This water dilutes the interior milieu and produces hyponatremia and hypo-osmolality. Water retention and dilutional hyponatremia develop months after the onset of sodium retention and ascites and are secondary to non-osmotic hypersecretion of AVP from the neurohypophysis in response to the reduced effective intravascular volume in cirrhosis[12,13]. Higher levels of AVP are responsible for water reabsorption in the distal collecting duct of the kidney. Water retention in patients with dilutional hyponatremia is a part of the positive fluid balance and contributes to the occurrence of ascites[12].

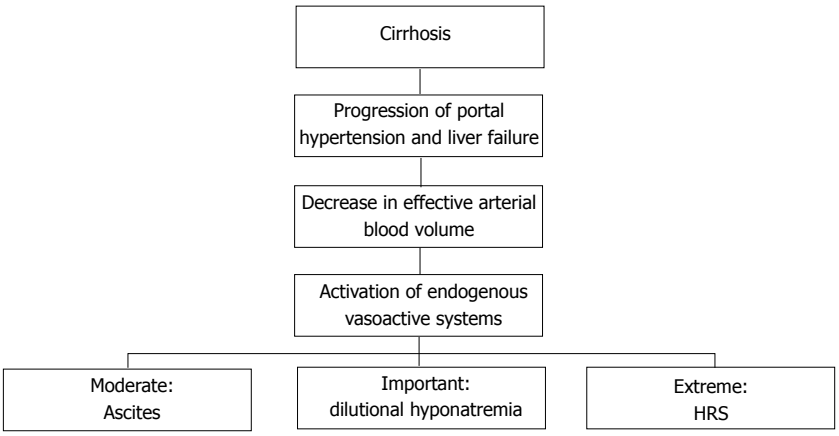

Figure 1 Hemodynamic alterations underlying the role of portal hypertension in the ascites, hyponatremia and hepato-renal occurrence in liver cirrhosis. HRS: Hepato-renal syndrome.

Interestingly, the renal synthesis of prostaglandin E2 is increased in cirrhotics to counteract the water-retaining effect of AVP and hence Non Steroid Anti-Inflammatory Drugs may worsen the renal excretion of solute-free water in these patients[14,15].

As previously stated, the clinical consequence of solute-free water excretion impairment is the development of hyponatremia. This type of hyponatremia is referred to as *dilutional hyponatremia* because it occurs in the setting of increased total body water and dilution of extracellular fluid volume.

Hyponatremia in cirrhosis and ascites has gained attention as a strong prognostic marker, particularly in patients awaiting liver transplantation, given that several reports indicate that when serum sodium concentration if combined with the Model for End-Stage Liver Disease (MELD) score improves the prognostic accuracy of MELD in patients listed for orthotopic liver transplantation[16-18].

In most patients the degree of hyponatremia is mild and the condition is asyntomatic and needs no specific therapy.

Renal vasoconstriction is the renal functional abnormality that develops later in patients with cirrhosis and ascites[19,20].

The occurrence of renal vasoconstriction in patients with cirrhosis and ascites is clinically relevant for several reasons. First, a significant proportion of these patients have refractory ascites, as sodium and water excretion are markedly impaired. Second, it predisposes to the development of HRS[21].

An increased activity of vasoconstrictor factors (mainly plasma renin activity and norepinephrine) and reduced activity of renal vasodilator factors acting on the renal circulation play the most important role in the pathogenesis of HRS because renal vasoconstriction in cirrhosis occurs in the absence of morphologic changes in the kidney[22].

The pathogenesis of renal vasoconstriction in cirrhosis is also related to changes in systemic hemodynamics. The most accepted theory considers renal vasoconstriction as the consequence of the extreme underfilling of the systemic arterial circulation due to marked vasodilatation of the splanchnic circulation, which activates homeostatic vasoconstrictor systems, whose effect on the kidney vasculature cannot be counterbalanced by either renal or systemic vasodilators[23,24].

Progression of functional renal alterations paralleled with cirrhosis course is shown in Figure 2.

VASOPRESSIN RECEPTOR ANTAGONISTS

Due to the aforementioned circulatory dysfunctions and activation of neuro-humoral systems leading to sodium and water retention, there has been an increasing interest in research on drugs that may improve circulatory and renal function in cirrhotics with refractory ascites and/or hyponatremia. Among such drugs, many affords have been sustained in developing and testing selective antagonists of the V2-receptors of vasopressin, known as vaptans.

AVP is a neuropeptide hormone synthesized by two hypothalamic nuclei (supraoptic and paraventricular nuclei) and secreted by the posterior pituitary in response to an increase in plasma tonicity or decrease in plasma volume[25].

The actions of AVP are mediated by three receptor subtypes: V1a, V1b and V2, all of them being G protein-coupled receptors and classified by their location[26]. V1a receptors are present on vascular smooth muscle cells, myocardium, platelets, and hepatocytes, and mediate vasoconstriction, platelet aggregation, and glycogenolysis[25,27,28]. V1b receptors have little selective distribution in the central nervous system. V2 receptors are expressed in principal cells of the renal collecting duct system. As shown in Figure 3, they mobilize intracellular vesicles of aquaporin 2 to the apical plasma membrane of collecting duct cells causing an increase in the reabsorption of free water. AVP acts on V2 receptors on the basolateral surface of principal cells resulting in activation of adenylyl cyclase. This leads to protein kinase activation resulting in preformed cytoplasmic vesicles called aquaporins getting inserted into the luminal membrane. They span the luminal membrane and permit movement of water down an osmotic gradient. The water absorbed is returned to the systemic circulation across the basolateral membrane. When the effect of AVP has worn off, water channels are removed from the luminal membrane by endocytosis, aggregate within clathrin-coated pits, and are returned to

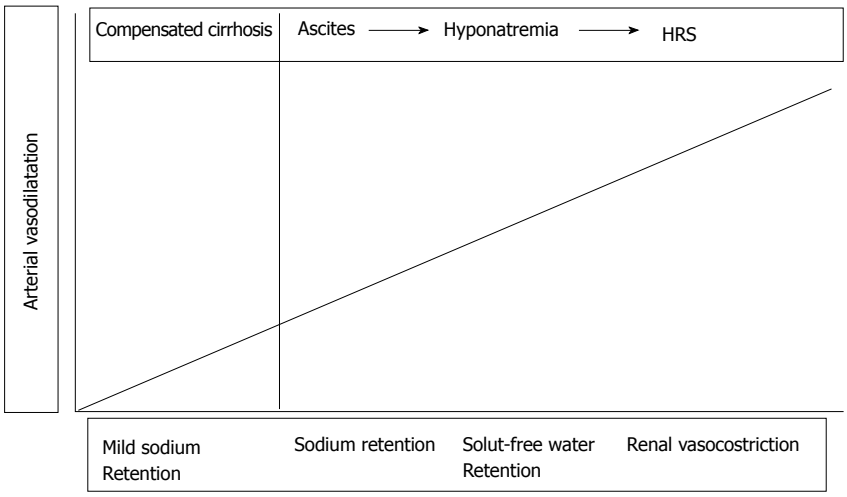

Figure 2 Progression of the alterations of renal functionality in cirrhotics. HRS: Hepato-renal syndrome.

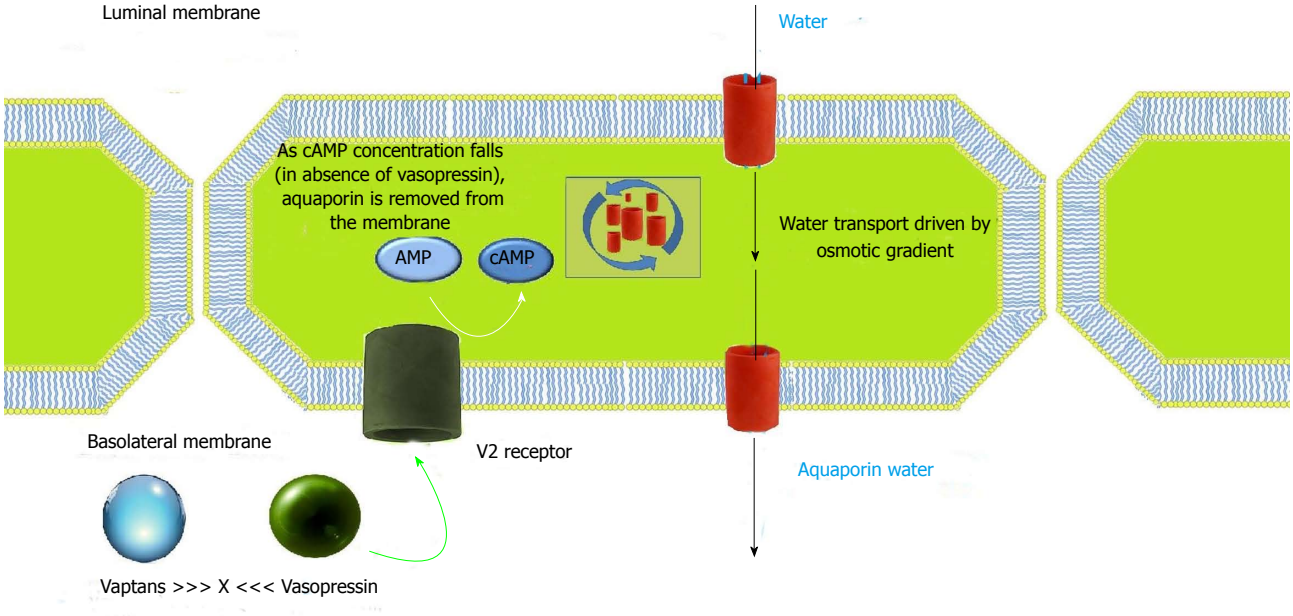

Figure 3 Mechanism of action of vaptans. In physiologic conditions, binding of arginine vasopressin to its receptor, raises introcytoplasmatic levels of cyclic adenosine monophosphate, thus resulting in increased expression of aquaporin on the luminal membrane of principal cells in collecting duct. Therefore, free water move down gradient *via* aquaporin from ductal lumen to blood. Vaptans, by blocking binding of arginine vasopressin to its receptor, inhibit water reabsorption. cAMP: Cyclic adenosine mono-phosphate.

the cytoplasm[29].

Orally and intravenously active non-peptide vasopressin receptor antagonists are called vaptans. They cause aquaresis, that is, excretion of solute-free urine (Figure 3). They differ from the diuretics as they promote excretion of water without the loss of electrolytes and hence are categorized as aquaretics.

USE OF VAPTANS IN THE MANAGEMENT OF REFRACTORY ASCITES

Two phase-2 studies have recently tested satavaptan associated to diuretics finding a therapeutic benefit[30,31]. Such result was confirmed in the setting of ascites recurrence after large-volume paracentesis (LVP) in another phase-2 study[32]. Unfortunately phase-3 randomized, placebo-controlled studies found a non-superiority and a worse safety profile of satavaptan in combination with diuretics in ascitic patients with even an increased morbidity and mortality for unknown reasons[33].

Therefore, both European and American guidelines suggest as first-line therapy of refractory ascites repeated LVPs + albumin (8 g/L of ascites removed) and Transjugular Intrahepatic Porto-Systemic Shunt as rescue therapy for patients with very frequent requirement of LVPs[34,35]. Main characteristics of vaptans recently tested in clinical trials are reported in Table 1.

USE OF VAPTANS IN THE MANAGEMENT OF DILUTIONAL HYPONATREMIA

Due to the aforementioned prognostic impact of hyponatremia in cirrhotic patients and the well-known association between low serum sodium and comorbidities (neu-

Table 1 Vaptans tested in trials

Name	Receptor selectivity	Administration	Dose (mg)	Half-life (h)
Conivaptan	$V_{1a}R/V_2R$	Oral, IV	40-80	31-78
Tolvaptan	V_2R	Oral	15-60	6-8
Lixivaptan	V_2R	Oral	50-400	7-10
Satavaptan	V_2R	Oral	5-25	14-17
Mozavaptan	V_2R	Oral	30-60	-

IV: Intravenous.

rological complications, above all), in the last years many efforts in order to define an effective and safe therapeutic algorithm for dilutional hyponatremia have been made.

The main therapy of hyponatremia, consisting of increasing solute-free water excretion, has recently been implemented with the introduction of vaptans[36,37]. A number of evidences show that a short-therapy with vaptans (1 wk to 1 mo) ameliorates solute-free water excretion and leads to the increase in serum sodium levels in 45%-82% of patients without particular side effects on renal function, urine sodium, circulatory function, and activity of RAAS[38-41].

Thirst is the main complication related to vaptans. Other possible consequences of vaptan use in cirrhotics are hypernatremia, dehydration, kidney failure, and osmotic demyelination syndrome due to an unregulated increase in serum sodium levels. On the other hand, these concerns found little confirm in the aforementioned studies and their low frequency makes such considerations more theoretical assumptions than real problems[38-41]. Nevertheless, in light of these reported complications, therapy with vaptans should always be started under medical control with an "in-hospital" regime and serum sodium shouldn't increase of more than 8-10 mmol/L per day[34]. Furthermore, vaptan therapy should be avoided in individuals affected by encephalopathy or who cannot guarantee an appropriate uptake of water due to the risk of dehydration and hypernatremia. The metabolism of these drugs is on charge of hepatic CYP3A enzymes; therefore, an unexpected increase in hematic vaptan levels could be due to drugs or compounds known as strong inhibitors of CYP3A such as ketoconazole, grapefruit juice, and clarithromycin. On the other hand, inducers of the CYP3A system, such as rifampicin, barbiturates, and phenytoin, may lead to a severe impairment of vaptan efficacy.

Tolvaptan was approved in the United States and Europe for the treatment of severe hypervolemic hyponatremia (< 125 mmol/L) due to SIADH, while for other conditions such as cirrhosis, ascites, and heart failure, only the American Food and Drug Administration (FDA) had licensed the drug.

Tolvaptan should be started with 15 mg/d and titrated progressively to 30 and 60 mg/d, if needed, following the serum sodium concentration. In the above reported studies, concerns raised only for some reported cases of gastrointestinal bleeding. Except for the aforementioned event, the safety profile of the drug resulted acceptable but clinicians should be aware that robust long-term data are lacking. However, in a recent placebo-controlled and open-label extension study of chronically administered tolvaptan in patients with autosomal dominant polycystic kidney disease, three cases of serious liver injury attributed to tolvaptan were observed[42]. Therefore, in a recently published safety announcement, FDA has forbidden the use of tolvaptan in patients with underlying liver disease, including cirrhosis, because the ability to recover may be impaired[43].

Conivaptan has also been licensed by FDA for the short term (5 d) intravenous treatment of hypervolemic hyponatremia but in cirrhosis data form randomized trials are lacking.

Given the narrow therapeutic window of these drugs, current practical guidelines state that management of symptomatic hyponatremia relies on infusion of saline and removal of the underlying etiologic mechanism (usually due to diuretic therapy)[34,35]. Vaptan therapy should be reserved to cases of severe hypervolemic hyponatremia (< 125 mmol/L) and should be introduced under careful medical monitoring in hospital regime. Patients may be discharged when sodium levels' stabilization is reached and no further adjustments of vaptan dose are needed. The proper length of therapy with vaptans is still unclear and concerns raise for long-term courses (> 1 mo)[34].

CONCLUSION

Vaptans represent a modern and promising therapeutic tool in the management of hydroelectrolytic imbalances in cirrhotic patients. Their safety profile and efficacy need further validation by randomized controlled trials.

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TOPIC HIGHLIGHT

WJH 6th Anniversary Special Issues (7): Nonalcoholic fatty liver disease

Pathogenesis and therapeutic approaches for non-alcoholic fatty liver disease

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Abstract

Non-alcoholic fatty liver disease affects approximately one-third of the population worldwide, and its incidence continues to increase with the increasing prevalence of other metabolic disorders such as type 2 diabetes. As non-alcoholic fatty liver disease can progress to liver cirrhosis, its treatment is attracting greater attention. The pathogenesis of non-alcoholic fatty liver disease is closely associated with insulin resistance and dyslipidemia, especially hypertriglyceridemia. Increased serum levels of free fatty acid and glucose can cause oxidative stress in the liver and peripheral tissue, leading to ectopic fat accumulation, especially in the liver. In this review, we summarize the mechanism underlying the progression of hepatic steatosis to steatohepatitis and cirrhosis. We also discuss established drugs that are already being used to treat non-alcoholic fatty liver disease, in addition to newly discovered agents, with respect to their mechanisms of drug action, focusing mainly on hepatic insulin resistance. As well, we review clinical data that demonstrate the efficacy of these drugs, together with improvements in biochemical or histological parameters.

Key words: Non-alcoholic fatty liver disease; Insulin resistance; Drugs; Pathogenesis

Core tip: In this review, we summarize the pathogenesis underlying the progression of hepatic steatosis to steatohepatitis and cirrhosis. We also discuss established drugs that are already being used to treat non-alcoholic fatty liver disease, in addition to newly discovered agents, with respect to their mechanisms of drug action, focusing mainly on hepatic insulin resistance. As well, we review clinical data that demonstrate the efficacy of these drugs, together with improvements in biochemical or histological parameters. Furthermore, we introduced future treatment option for non-alcoholic fatty liver disease.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD), the accumulation of lipid within hepatocytes, is a common disease[1]. The worldwide prevalence of NAFLD is estimated to be 20%-30%[2], although increasing to 57%-74% among obese patients[3]. NAFLD refers to a wide spectrum of fatty degenerative disorders of the liver in the absence of alcohol intake, ranging from simple steatosis to steatohepatitis and cirrhosis[4]. Nonalcoholic steatohepatitis (NASH) is histologically characterized by inflammatory cell recruitment. NASH is a significant risk factor for hepatic cirrhosis, compared with simple steatosis[5], and 4%-27% of cases of NASH progress to hepatocellular carcinoma after the development of cirrhosis[6]. In one study, NAFLD was pres-

ent in 75% of obese [body mass index (BMI) ≥ 30 kg/m^2] patients, 16% of non-obese patients, and 34%-74% of patients with type 2 diabetes[7]. Another study reported diagnoses of fatty liver in 39% of obese (BMI ≥ 30 kg/m^2) patients, 41% of patients with known type 2 diabetes, and 32% of patients with dyslipidemia[8]. Patients with NAFLD are not only insulin resistant, but also tend to present with alterations in plasma triglyceride levels[9]. NAFLD is strongly associated with metabolic syndrome, especially insulin resistance, central obesity, and dyslipidemia. Therefore, NAFLD is regarded as a difficult to treat component of metabolic syndrome[10]. In this review, we investigate the mechanisms of hepatic fat accumulation, focusing on the role of insulin resistance therein, and review current therapeutic options and new candidate drugs for the treatment of NAFLD.

PATHOGENESIS

Insulin resistance - free fatty acid flux and hyperinsulinemia

Hepatic steatosis is caused by an imbalance in triglyceride movement through the liver cell. Triglyceride is composed of free fatty acid (FFA) and glycerol. Total FFA is derived from three sources, the diet (15%), *de novo* synthesis (26%), and circulating FFA (56%)[11]. A high-fat diet is known to lead to the development of hepatic steatosis. However, estimates suggest that approximately 60% of liver fat is derived from circulating nonesterified fatty acids (NEFAs) in individuals who eat a normal fat-containing diet[11]. Obesity is associated with insulin resistance and an elevated leptin level. In particular, increased visceral fat correlates with peripheral and hepatic insulin resistance[12,13]. Insulin resistance in skeletal muscle and adipose tissue results in increased levels of NEFAs through increased lipid oxidation in adipose tissue (Figure 1). Accordingly, NEFA flux plays an important role in hepatic fat accumulation[14]. An increase in hepatocellular diacylglycerol is associated with decreased tyrosine phosphorylation of insulin receptor substrate 2 (IRS-2)[15,16]. In turn, the decreased activity of IRS-2 and PI3K leads to increased hepatic glucose production[17]. Hyperinsulinemia also arises in response to insulin resistance in adipose tissue, leading not only to downregulation of IRS-2 in the liver, but also to a continued increase in the level of sterol regulatory element binding protein-1c (SREBP-1c) *via* the insulin signaling pathway involving AKT2, liver X receptor (LXR) and mammalian target of rapamycin[18,19]. Elevated levels of SREBP-1c up-regulate lipogenic gene expression, increase fatty acid synthesis, and accelerate hepatic fat accumulation[20]. Additionally, overexpression of SREBP-1c represses IRS-2 expression[21]. Glucose-stimulated lipogenesis is mediated by carbohydrate-responsive element-binding protein (ChREBP) in the liver. Like SREBP-1c, ChREBP increases lipogenesis by inducing lipogenic gene expression during consumption of a diet high in carbohydrates[22,23].

Endoplasmic reticulum stress

The endoplasmic reticulum (ER) is an intracellular organelle that plays an important role in the synthesis, folding, and trafficking of proteins. Cellular nutrient status and energy condition highly influence the function of the ER, and dysfunction in the ER causes accumulation of unfolded proteins therein, triggering an unfolded protein response (UPR)[24]. Under stress, such as hypoxia, inflammation and energy excess, UPR is characterized by adaptive cellular processes of increased degradation of proteins and translational arrest of protein synthesis to restore normal function of the ER. As well, UPR mediates metabolic and immune responses that aggravate insulin resistance[25-27]. Both PKR-like kinase and the α-subunit of translation initiation factor 2 (eIF2α), well-known ER stress markers, are increased in hepatocytes of ob/ob mice, compared with control mice[26]. Obesity causes ER stress that leads to suppression of insulin signaling through serine phosphorylation of insulin receptor substrate-1 (IRS-1) and activation of the c-Jun N-terminal kinase (JNK) pathway[26]. Among subjects with metabolic syndrome, those with NASH showed higher levels phosphorylated JNK protein, compared to subjects with simple hepatic steatosis. Furthermore, subjects with NASH did not generate spliced manipulation of X-box–binding protein-1 (sXBP-1), which is a key regulator in ER stress in relation to insulin action[24,26]. Additionally, weight reduction in obese subjects has been shown to induce improvement in ER stress *via* suppression of phosphorylated JNK and eIF2α in adipose tissue and the liver[28].

Role of oxidative stress - mitochondrial dysfunction

The two-hit hypothesis is a key concept of NAFLD pathogenesis. In fatty livers, simple hepatic steatosis (first hit) sensitizes the liver to inflammatory cytokines or oxidative stress (second hit), leading to development of steatohepatitis[29]. Oxidative stress is resulted from a serious imbalance between the limited antioxidant defenses and excessive formation of reactive species such as reactive oxygen species (ROS) or reactive nitrogen species (RNS)[30]. ROS is an integrated term that describes a variety of species of free radicals derived from molecular oxygen, such as superoxide, hydrogen peroxide, and hydroxyl[31]. In cells, mitochondria are a major source of ROS generation. The important factor modulating mitochondrial ROS generation is the redox state of the respiratory chain[32,33]. FFAs are metabolized *via* the mitochondrial β-oxidation pathway and the tricarboxylic acid (TCA) cycle, which generates citrate that in turn inhibits glycolysis. As a result, glucose oxidation and glucose uptake *via* glucose transporter type 4 (GLUT4) in skeletal muscle are reduced[34,35]. To compensate for the excessive fat storage in the liver, increased hepatic FFA uptake stimulates hepatic oxidation of fatty acids in obese individuals. Mitochondrial FFA oxidation is maintained until mitochondrial respiration becomes severely impaired[36,37]. However, accelerated β-oxidation not only causes excessive electron flux in the electron transport chain, but also leads to increased production of ROS, and can lead to mitochondrial dysfunction[38]. Excessive ROS production by mitochondria can lead to oxidative damage to

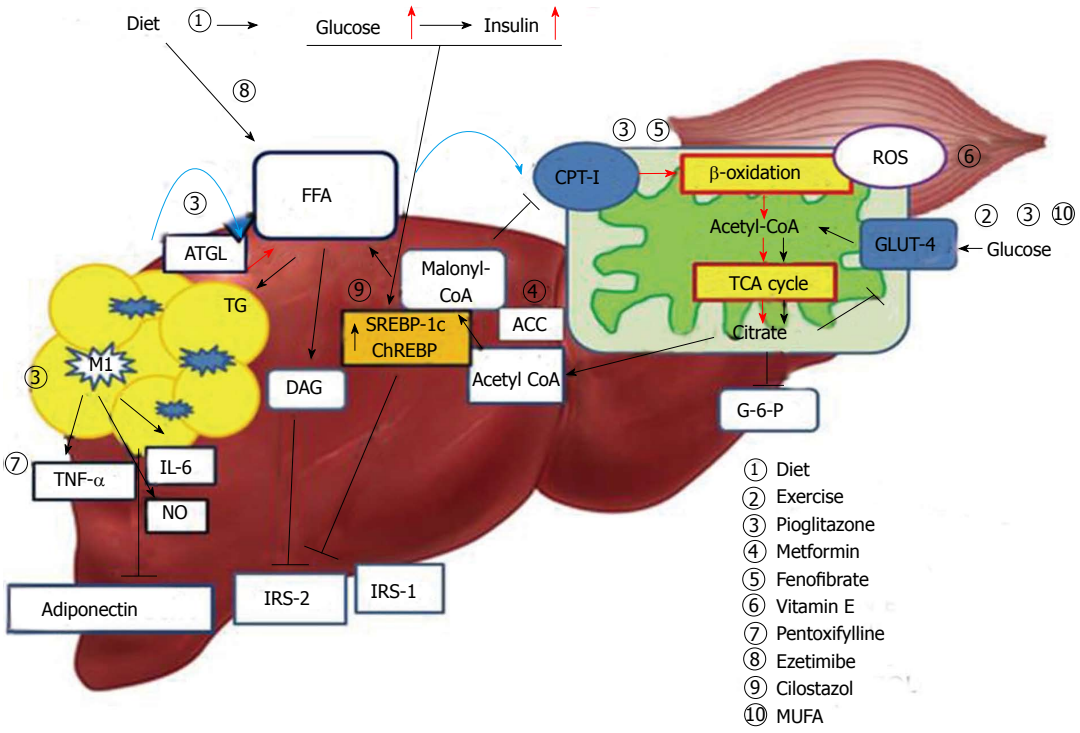

Figure 1 Mechanism of hepatic insulin resistance and the key pathway of drug action. Delivery of FFAs to the liver and skeletal muscle is increased in insulin resistance conditions, and these are metabolized *via* mitochondrial β-oxidation. Consequently, hyperglycemia and increased hepatic FFA uptake reduce glucose uptake and oxidation in skeletal muscle. Diet and exercise are the main treatment strategies for this pathogenesis; insulin sensitizers and MUFA may contribute to reducing peripheral insulin resistance. Pioglitazone and fenofibrate act on β-oxidation of mitochondria and reduce hepatic steatosis. Accelerated β-oxidation also causes increased production of ROS. Vitamin E can reduce oxidative stress. Adipose tissue inflammation of the liver leads to inflammatory activation of hepatic Kupffer cells via classic response (M1) and produce inflammatory cytokines. This is also associated with decreased adiponectin levels and promotes hepatic steatohepatitis. Pentoxifylline inhibits TNF-α and alleviates steatohepatitis. Hyperglycemia caused by insulin resistance up-regulates lipogenic gene expression, such as SREBP-1c and ChREBP, and induces lipogenesis in hepatocytes. Cilostasol may inhibit SREBP-1c. FFA: Free fatty acid; TG: Triglyceride; CPT- Ⅰ: Carnitine palmitoyltransferase-Ⅰ; ACC: Acetyl-CoA carboxylase; ATGL: Adipose triglyceride lipase; ChREBP: Carbohydrate responsive element binding protein; SREBP-1c: Sterol regulatory element binding protein-1c; TCA: Tricarboxylic acid; ROS: Reactive oxygen species; IRS: Insulin receptor substrate; DAG: Diacylglycerol; G-6-P: Glucose 6-phosphate; TNF-α: Tumor necrosis factor- α; MUFA: Monosaturated fatty acids; M1: Kupffer cells activated *via* classic pathway.

the mitochondrial membrane and DNA and can impair mitochondrial metabolic functions[33]. The increase in hepatic lipogenesis in NASH results in increased production of malonyl-CoA. Inhibition of carnitine palmitoyltransferase-I (CPT-1) by malonyl-CoA leads to decreased entry of long chain fatty acid into the mitochondria, and causes reduced β-oxidation and enhanced triglyceride accumulation in the liver[38-40]. The nuclear receptor peroxisome proliferator-activated receptor α (PPAR-α) plays an important role in the transcriptional control of many enzymes involved in mitochondrial fatty acid β-oxidation. Peroxisome proliferator-activated receptor-gamma coactivator (PGC) -1α cooperates with PPAR-α and regulates genes that encode mitochondrial fatty acid oxidation enzymes, such as CPT-1 and medium chain acyl-CoA dehydrogenase[40]. Previously, a PPAR-α-deficient mouse model showed a lack of hepatic peroxisome proliferation and dyslipidemia with obesity and hepatic steatosis[41].

Inflammation and adipokines

Overall obesity is correlated with NAFLD, and accumulation of intra-abdominal fat in particular is believed to play an important role in the development of insulin resistance[12,13]. Meanwhile, hepatic fat accumulation is associated with insulin resistance independent of intra-abdominal fat accumulation and overall obesity. Even in normal weight subjects, hepatic steatosis has been shown to be related to various parameters of insulin resistance, such as basal glucose level or serum FFA level[42]. In addition to being a major organ of triglyceride deposition, adipose tissue acts an endocrine organ that secretes several hormones[43]. Adipocytes secrete adiponectin and leptin, in addition to the other adipokines, such as retinol-binding protein, tumor necrosis factor-α (TNF-α), interleukin 6 (IL-6), and plasminogen activator inhibitor-1[43]. Adiponectin stimulates phosphorylation of AMP-activated protein kinase (AMPK) and acetyl-CoA carboxylase (ACC) in the liver and muscles, thereby, increasing glucose utilization and fatty-acid oxidation[44]. In a previous study, serum adiponectin levels decreased with an increases in obesity, in particular increases in intra-abdominal fat mass[45,46]. In another study, adiponectin knockout mice fed a high-fat diet exhibited increased incidences of obesity, hyperinsulinemia, and steatohepatitis. These experimental data indicate that adiponectin may play a key protective role against the progression of NASH[47]. Reportedly, adipose tissue in obese individuals stimulates a shift in macrophage activation from the

Table 1 Treatment outcomes of variable regimens

Study	Treatment group	Control group	No.	Study design	Duration (mo)	Histology	Liver enzymes	US
Life style modification								
Huang *et al*[66]	Diet	-	12	Open label	12	Improved	-	-
Ueno *et al*[65]	Diet/Exercise	Control	15	Open label	3	Improved	Improved	-
Pioglitazone (insulin sensitizer)								
Promrat *et al*[71]	Pioglitazone	-	18	Open label	12	Improved	Improved	-
Belfort *et al*[72]	Pioglitazone and Diet	Placebo	55	RCT	6	Improved	Improved	-
Aithal *et al*[73]	Pioglitazone and diet/Exercise	Placebo	74	RCT	12	Improved	Improved	-
Sanyal *et al*[74]	Pioglitazone	Placebo	163	RCT	24	Improved	Improved	-
Metformin (insulin sensitizer)								
Garinis *et al*[100]	Metformin and Diet	Diet	50	RCT	6	-	-	Improved
Haukeland *et al*[103]	Metformin	Placebo	48	RCT	6	-	-	-
Uygun *et al*[101]	Metformin and Diet	Control	50	Open label	6	-	-	Improved
Bugianes *et al*[99]	Metformin	Diet	53	RCT	12	Improved	Improved	-
Bugianes *et al*[99]	Metformin	Vitamin E	57	RCT	12	-	Improved	-
Vitamin E (antioxidant)								
Bugianesi *et al*[99]	Vitemin E	Diet	55	RCT	12	-	-	-
Sanyal *et al*[74]	Vitamin E	Placebo	167	RCT	24	Improved	Improved	
Vajro *et al*[109]	Vitamin E	Diet	25	RCT	6	-	-	-
Other drugs								
Sanjay *et al*[112]	Pentoxifylline	-	18	Open label	6	-	Improved	-
Yoneda *et al*[127]	Ezetimibe	-	10	Open label	6	Improved	Improved	
Vasilios *et al*[118]	Statin	Control	437	Open label	36	-	Improved	-
Lindor *et al*[130]	UDCA	Placebo	166	RCT	48	-	-	-
Capani *et al*[138]	PUFA	Control	42	RCT	12	-	Improved	Improved

No.: Number; US: Ultrasonography; RCT: Randomized controlled trial.

alternative response (M2) to the classic response (M1), and these classically activated macrophages secrete a variety of inflammatory cytokines, such as TNF-α, IL-6, and NO[48]. Additionally, studies showed that inflammatory activation of hepatic Kupffer cells in ob/ob mice promotes hepatotoxicity, resulting in hepatic insulin resistance and steatohepatitis[49,50]. Thus, increases in TNF-α and IL-6 in obese subjects may play an important role in insulin resistance and hepatic steatosis[51,52].

Gut-microbial alternation and TLRs stimulation

As mentioned above, obesity is often associated with NASH and systemic inflammation characterized increases in inflammatory cytokine levels. Obesity also can cause increased intestinal mucosa permeability and endotoxin levels in portal circulation that can contribute to hepatocellular damage[53,54]. Kupffer cells in the liver play a key role in clearing endotoxin and are activated through Toll like receptor 2,3,4 and 9 signaling in the presence of endotoxin. In particular, activation of Toll like receptor4 (TLR4) is reportedly associated with stimulation of lipopolysaccharide (LPS)[55-57]. Previously, animal model studies showed that TLRs 2, 4 and 9 may contribute to the pathogenesis of NAFLD[55,58]. Activated Kupffer cells induce expression of pro-inflammatory cytokines, such TNF-α, IL-6, IL-18 and IL-12 as well as anti-inflammatory cytokines[59]. TLRs including TLRs 2,4 and 9 are activated *via* a MyD88 dependent pathway. This pathway consists of the activation of serine kinase IL-1R-associated kinase and TBF-receptor-associated factor 6 and is involved in the activation of the transcription factor NF-κB, which is related to inflammatory cytokine production[60].

TREATMENT

Life style modification - diet and exercise

Weight loss due to diet and exercise has been demonstrated to alleviate hepatic steatosis[61]. Body weight reduction and exercise are important independent factors for improvement of hepatic steatosis[62]. In obese women, hepatic fat content measured by magnetic resonance imaging was shown to decrease in response to weight loss interventions[63]. Several studies have shown a significant reduction in alanine transaminase (ALT) levels and improvement in biochemical markers following intervention with a calorie-restricted diet combined with exercise[63,64]. A few studies have also shown histologic improvement with increased exercise and weight reduction[65,66] (Table 1). Exercise improves insulin sensitivity in skeletal muscle *via* GLUT4 expression and increases glucose utilization. Thus, exercise decreases levels of serum glucose and insulin[67]. An improvement in hyperinsulinemia can result in decreased liver fat mass, because hyperinsulinemia stimulates hepatic steatosis *via* the SREBP-1c pathway[19]. In particular, NAFLD patients with metabolic syndrome show a great improvement in hepatic steatosis after weight loss[68].

Insulin sensitizer-thiazolidinedione, metformin

Thiazolidinedione: Thiazolidinediones (TZDs) are insulin-sensitizing agents that have been shown to improve not only hepatic steatosis, but also whole body insulin resistance[69]. Improvements in insulin resistance and histologic and biochemical parameters were reported with TZD treatment[70-74]. Rosiglitazone is one TZD and

is associated with an increased risk of myocardial infarction and cardiovascular death[75]. Meanwhile, pioglitazone is regarded as safe in regards to cardiovascular outcomes and is not associated with increased cardiovascular risk[76,77]. In patients with type 2 diabetes, pioglitazone has been recommended for the treatment of steatohepatitis proven by liver biopsy; however, its role in non-diabetic patients has not been established. The American Association for the Study of Liver Disease (AASLD) introduced pioglitazone as a first-line treatment of NAFLD in patients with type 2 diabetes[78]. TZDs increase glucose utilization of peripheral tissue and improve whole body insulin sensitivity as measured by the hyperinsulinemic euglycemic clamp technique, in patients with type 2 diabetes. Moreover, serum adiponectin levels increase and serum insulin levels decreases after treatment with pioglitazone[79,80]. An increase in serum adiponectin contributes to alleviation of hepatic steatosis and improves hepatic and peripheral insulin resistance[79]. As mentioned above, adiponectin increases lipid oxidation of FFA by ACC phosphorylation in the liver[44], and promotes the activation of anti-inflammatory M2 macrophages rather than M1 macrophages[81]. Obesity is closely related to an increase in NAFLD risk[82]. Increased levels of inflammatory adipose tissue macrophages (ATMs) and their secreted cytokines in a mouse model were shown to be related to systemic insulin resistance, which is associated with NAFLD development[15,83]. According to previous studies, ATMs are increased in obese subjects[84], and pioglitazone treatment results in not only a decrease in ATM content, but also in the inflammatory markers, TNF-α, IL-6, and inducible nitric oxide synthase[85,86]. TZDs also promote the alternative activation of monocytes into macrophages with anti-inflammatory properties, as opposed to the pro-inflammatory phenotype[87]. Although the pathogenesis of NAFLD development is closely related to obesity, the distribution of fat is more important than overall obesity. Excessive visceral fat accumulation plays an important role in the development of insulin resistance and NAFLD by acting as a source of FFA[12]. Pioglitazone is strongly associated with fat redistribution, increases in subcutaneous fat area decreases in visceral fat area (visceral to subcutaneous fat ratio)[88]. Another study showed that the ratio of visceral fat thickness to subcutaneous fat thickness decreases after pioglitazone treatment and is correlated with a change in high sensitivity C-reactive protein levels[89]. TZD treatment results revealed a decrease in serum FFA levels, which in turn reduced FFA supply to the liver and led to a decrease in hepatic triglyceride content[90]. Recent studies have focused on the role of sirtuin-6 (SIRT-6) in the glucose and lipid metabolism associated with TZDs. TZD treatment reduced hepatic fat accumulation and increased expression of SIRT-6 and PGC1-α in rat livers[91]. Also, liver-specific SIRT-6 knockout mice exhibited fatty liver formation[92], leading to NASH[93].

Metformin: Metformin improves insulin resistance and hyperinsulinemia by increasing peripheral glucose uptake and decreasing hepatic gluconeogenesis[94]. Metformin activates AMP kinase *via* a LKB-1 dependent mechanism in skeletal muscle. Also it can activate AMPK by stimulating AMP accumulation in hepatocytes. The increase in AMP interferes with glucagon action and decreases cAMP levels, leading to decreased production of hepatic glucose[95,96]. Activation of AMPK results in decreased hepatic triglyceride synthesis and increased fatty acid oxidation[97], as well as attenuated hepatic steatosis due to decreased SREBP-1c activity[98]. A randomized controlled trial showed that subjects treated with metformin exhibit significant improvement in ALT levels, compared with those who were on a restricted diet or were treated with vitamin E, as well as improvements in histology after a 12 mo of treatment[99,100]. Many studies have shown that metformin treatment normalizes transaminase levels and decreases hepatic steatosis as determined by follow-up ultrasound; nevertheless, histologic data remain limited[100-103]. As NASH is closely associated with development of HCC and liver fibrosis, metformin may be limited in the reduction of these severe outcomes, including mortality[104].

Antioxidant - vitamin E (α-tocopherol), pentoxifylline

As mentioned above, oxidative stress contributes to the progression of NASH from simple hepatic steatosis. A recent study reported that subjects who were treated with vitamin E (α-tocopherol) showed improvement in hepatic steatosis and serum aminotransferase levels compared to a placebo group[74]. Vitamin E (α-tocopherol) has been used to treat non-diabetic NASH patients diagnosed by liver biopsy[78]. Meta-analyses of vitamin E have revealed an increase in all-cause mortality with high dose (≥ 400 IU/d) vitamin E supplement use, especially in subjects with chronic disease or at high risk for cardiovascular disease events, such as type 2 diabetes. However, these results are uncertain in healthy subjects[105,106]. Two pilot studies reported improved ALT levels with vitamin E treatment[107,108]. However, two randomized controlled trials failed to show the efficacy of vitamin E treatment in NAFLD[109,110]. Pentoxifylline, a TNF-α inhibitor, has also been considered for the treatment of hepatic steatosis, since it plays an important role in the progression of simple hepatic steatosis to steatohepatitis. In previous studies, administration of pentoxifylline generated improvements in biochemical markers, such as aminotransferase and Homa-IR, in patients with NASH[111,112]. Nevertheless, further study is needed to prove the efficacy of pentoxifylline with respect to histologic improvement of NAFLD.

Lipid-lowering agents - fibrates, ezetimibe and statins

Hypertriglyceridemia is a major component of metabolic syndrome and is strongly associated with NAFLD. Increased FFA delivery to the liver causes accumulation of hepatic fat[9]. Many different lipid-lowering agents have been investigated for the treatment of NAFLD. Patients treated with gemfibrozil, one type of fibrate, showed decreased ALT levels, compared to the control group[113]. However, clofibrate did not show a beneficial effect on

NAFLD[114]. PPAR-α modulates not only FFA transport and β-oxidation to decrease triglyceride in hepatocytes, but also glucose and amino acid metabolism in liver and skeletal muscle. PPAR-α activation is involved in lipoprotein metabolism by increasing lipolysis, thus reducing the production of triglyceride-rich particles[115]. Fenofibrate increased levels of PPAR-α and decreased hepatic steatosis in an APOE2KI mouse model that represented diet-induced NASH[116]. A prospective study using atorvastatin reported significant reductions in serum transaminase level[117,118]. Atorvastatin induces hepatic low-density lipoprotein receptor-related protein 1 (LRP-1) that plays an important role in clearance of circulating triglyceride in the liver[119]. In disposal of chylomicron in hepatocytes, interaction of LRP-1 receptors and apolipoprotein E (ApoE) play important roles[120]. Thus, ApoE-deficient mice showed development of hepatic steatosis even when they were fed a normal chow-diet. Accordingly, ApoE may play a key role in intracellular metabolism and control of VLDL production by hepatocytes[121]. Statins are very important drugs to treat dyslipidemia in subjects with both insulin resistance and NAFLD. However, there is continued concern about the use of statins in subjects with established liver disease. According to several randomized controlled studies and retrospective studies, statin rarely induces serious liver injury[122-125]. Ezetimibe, a potent inhibitor of cholesterol absorption, has been reported to improve hepatic steatosis in obese Zucker fatty rats[126]. In a randomized controlled study, six months of treatment with ezetimibe led to improvements in serum ALT levels and histologic observations[127,128].

Ursodeoxycholic acid

Ursodeoxycholic acid (UDCA) is widely used in subjects with abnormal liver function. Several studies have investigated the efficacy of UDCA as a treatment drug of NAFLD, reporting that UDCA treatment attenuated hepatic steatosis, including histologic improvement[114,129,130]. However, in a placebo controlled, randomized control trial, UDCA exhibited limited efficacy in histologic improvement in subjects with NASH and improvements in liver enzyme did not differ in the UDCA group, compared to the placebo group[130]. Accordingly, AASLD does not recommend UDCA for the treatment of NAFLD[78].

Other treatment options - future candidates

Cilostazol: SREBP-1c is a key regulator of lipogenic gene expression in hepatocytes. Recent data have shown that cilostazol, a selective type Ⅲ phosphodiesterase inhibitor, inhibits SREBP-1c expression *via* the suppression of LXR and Sp1 activity[131]. Cilostazol also decreases serum triglyceride levels by increasing lipoprotein lipase (LPL) activity in STZ-induced diabetic rats[132]. Also, experimental data show that cilostazol stimulates LRP1 promoter activity in hepatocytes, leading to increased hepatic LRP1 expression[133]. In a study that used two experimental NAFLD models, both high-fat/high-calorie (HF/HC) diet mice and the choline-deficient/L-amino acid-defined (CDAA) diet mice, cilostazol generated improvement in hepatic steatosis in both mice models[134]. Cilostazol exhibits the potential for improvement of hepatic steatosis, and further data on its role in NAFLD are needed.

Polyunsaturated fatty acids and monounsaturated fatty acids: Polyunsaturated fatty acids (PUFAs) are found primarily in safflower, corn, soybean, cottonseed, sesame, and sunflower oils. Omega-3 fatty acids are representative of PUFA. A marked increase in long-chain PUFA n-6/n-3 ratio is observed in NAFLD patients and is associated with increased production of pro-inflammatory eicosanoids and dysregulation of liver and adipose tissue function[135]. PPAR-α activity is impaired in conditions in which levels of circulating n-3 PUFA are decreased and the n-6/n-3 fatty acid ratio is increased[136,137]. Treatment with n-3 PUFA was shown to improve biochemical parameters and alleviated hepatic steatosis by ultrasound follow-up[138,139]. Monounsaturated fatty acids (MUFAs) are comprised in olive oil. In a rat model, supplementation with MUFA resulted in improved insulin sensitivity, compared to rats fed a saturated fatty acid (SFA) diet. Additionally, GLUT4 translocation in skeletal muscle was decreased in rats fed a SFA diet, but not in those fed a MUFA diet. Increased GLUT4 translocation is related to an improvement in insulin sensitivity[140]. In obese rats, MUFA diet attenuated hepatic steatosis and altered hepatic fatty acid levels[141]. The beneficial effects of dietary MUFA in NAFLD patients should be investigated.

GLP-1 analogue: Exenatide is the synthetic form of exendin4 and it stimulates endogenous insulin secretion, leading to decreases in blood glucose. In one animal study, treatment of exendin4 resulted in a decrease of hepatic fat content, as well as reduction of fatty acid synthesis, in the liver of ob/ob mice[142]. In patients with type 2 diabetes, an exenatide treatment group showed greater improvements in liver enzymes, attenuating hepatic steatosis, than the metformin treatment group. However, this study had limitations of a lack of histologic confirmation of the liver[143]. To prove the efficacy of glucagon like peptide-1 (GLP-1) analogue in treatment of NAFLD, randomized controlled trials over a longer period are required.

MK615: MK615 is extracted from Japanese apricots, and can suppress the production of inflammatory cytokines such as TNF-α and IL-6 by inactivating NF-κB[144,145]. MK615 is regarded as a hepatoprotective agent, as it has been shown that a MK615 treatment group exhibited greater decreases in liver enzyme levels, compared with control groups. In rat models, MK615 treatment mice showed more improved liver histology than control mice[146]. Thus, further studies are required to clarify the effects of MK615 in subjects with NAFLD.

CONCLUSION

NAFLD is a common disease that can progress to liver cirrhosis. Moreover, NAFLD is strongly associated with type 2 diabetes and insulin resistance. NAFLD is the

result of complex interactions among diet, metabolic components, adipose tissue inflammation, and mitochondrial dysfunction. The pathogenesis of hepatic steatosis has not yet been fully determined. In this review, we outlined previously known mechanisms of NAFLD, as well as introduced new mechanisms that have been recently discovered. Above all, we reviewed the mechanisms of drugs matched to the pathogenesis of NAFLD. Furthermore, we introduced future treatment option for NAFLD. TZDs play a key role in restoring insulin sensitivity and decreasing adipose tissue inflammation, generating histologic improvements in hepatic steatohepatitis. Pioglitazone can be used to treat NASH in patients with type 2 diabetes with biopsy-proven NAFLD; meanwhile, non-diabetic patients can be treated with vitamin E. Metformin is a well-known insulin sensitizer; however, further study is needed to prove histologic improvements in patients with NAFLD. Additionally, the cholesterol-lowering agent ezetimibe has also shown histologic improvements. Cilostazol acts on SREBP-1c and can improve dyslipidemia; however, further research is needed to clarify the relationship between NAFLD and cilostazol. Finally, there is an outstanding need for effective preventive and therapeutic regimens to overcome NAFLD.

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MINIREVIEWS

Perceptions of post-transplant recidivism in liver transplantation for alcoholic liver disease

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Abstract

Although alcoholic liver disease (ALD) is regarded as a common indication for liver transplantation (LT), debatable issues exist on the requirement for preceding alcoholic abstinence, appropriate indication criteria, predictive factors for alcoholic recidivism, and outcomes following living-donor LT. In most institutions, an abstinence period of six months before LT has been adopted as a mandatory selection criterion. Data indicating that pre-transplant abstinence is an associated predictive factor for alcoholic recidivism supports the reasoning behind this. However, conclusive evidence about the benefit of adopting an abstinence period is yet to be established. On the other hand, a limited number of reports available on living-donor LT experiences for ALD patients suggest that organ donations from relatives have no suppressive effect on alcoholic recidivism. Prevention of alcoholic recidivism has proved to be the most important treatment after LT based on the resultant inferior long-term outcome of patients. Further evaluations are still needed to establish strategies before and after LT for ALD.

Key words: Abstinence; Alcoholic liver disease; Liver transplantation; Six-month rule

Core tip: Prevention of alcoholic recidivism has proved to be the most important treatment after liver transplantation based on inferior long-term outcome of patients. Further evaluations, however, are still needed to establish strategies before and after liver transplantation with alcoholic liver diseases.

Kawaguchi Y, Sugawara Y, Akamatsu N, Kaneko J, Tanaka T, Tamura S, Aoki T, Sakamoto Y, Hasegawa K, Kokudo N. Perceptions of post-transplant recidivism in liver transplantation for alcoholic liver disease. *World J Hepatol* 2014; 6(11): 812-817 Available from: URL: http://www.wjgnet.com/1948-5182/full/v6/i11/812.htm DOI: http://dx.doi.org/10.4254/wjh.v6.i11.812

INTRODUCTION

Alcoholic liver disease (ALD) is regarded as a common indication for liver transplantation (LT), and accounts for approximately 40% of all primary transplants in Europe[1] and 25% in the United States[2]. One of the reasons making LT for ALD a complicated topic of issue is that alcohol abuse is the primary cause for end-stage liver disease development. Patients themselves are viewed as being responsible for their illness as compared to other diseases including cholestatic liver diseases and viral cir-

rhosis. Thus, controversy may exist over organ allocation to ALD patients in deceased-donor liver transplantation (DDLT). Organ allocation to patients with self-inflicted disease is less acceptable to society[3-5], and post-transplant alcoholic recidivism may raise questions on sharing organs as a public resource. By contrast, living-donor liver transplantation (LDLT), which remains the mainstream approach in Asia including Japan, does not conflict with the above-mentioned issues on organ allocation. However, requiring an abstinence period of at least six months (the so-called six-month rule[6]) to soften the controversy may also be debatable because the benefit of such pre-transplant abstinence remains unclear. Nevertheless, prevention of alcoholic recidivism is inevitably the most important factor to enhance medical benefits of LT and to gain more public acceptance as well. In the present article, we review the current status of LT for ALD mainly derived from DDLT cases, and focus on controversies involved in LDLT with the aim to explore the future direction of LT for ALD.

LT FOR ALD

Selection criteria

Selection criteria of LT for ALD, such as pre-transplant abstinence period, participation in rehabilitation program, and consultation with a psychiatrist, have been used in most institutions in addition to common criteria for other original diseases[7-13]. This is presumably because the criteria allow observations needed to determine the recovery odds from potential liver failure[7,14,15] and prevent post-transplant alcoholic recidivism[16-20]. In addition, there is a preponderance of evidence supporting that a pre-transplant abstinence period of six months has become a mandatory selection criterion[8,11-13,19-21], as its benefit was reported by Bird *et al*[6] in 1990. However, there are also reports indicating that an abstinence period of more than six months is not a significant predictive factor for alcoholic recidivism[22-24], along with those demonstrating that LT candidates with ALD barely survive for six months even with no alcohol intake[15,23]. A solid validation for requiring pre-transplant abstinence, as well as optimal duration of abstinence, if necessary, has yet to be established.

Alcoholic recidivism

Alcoholic recidivism has been considered to negatively impact postoperative compliance and long-term outcomes of recipients[21,24-30]. This perception may have encouraged LT professionals to evaluate predictive factors for alcoholic recidivism and therefore, to require specific criteria for ALD patients to prevent alcoholic recidivism in addition to commonly applied criteria. Rates and predictive factors of alcoholic recidivism are summarized according to the previous reports in Table 1[11,19-22,24,31,32]. The rates of alcoholic recidivism ranged widely from 10% to 42% as a result of inconsistent definitions on alcoholic recidivism and follow-up time. In fact, DiMartini *et al*[33] classified post-transplant alcohol consumption patterns into five categories based on time until relapse, three of which are harmful to the patients: no alcohol use, infrequent/low level of consumption, early onset/moderate and decreased consumption, later onset/harmful level of consumption, and early onset/heavy/increasing consumption. According to this classification, 46% of patients developed alcohol recidivism, with harmful use of alcohol accounting for 19%. In addition to inconsistent definitions on alcoholic recidivism, the fact that its detection is mainly based on statements from patients and/or reports from relatives makes evaluation difficult[11,19-22,24,31,32,34,35]. Random conducting of blood alcohol tests is useful for surveillance of ALD patients[19] as indicated through the resulting reduced rate of pre-transplant recidivism. With respect to predictive factors for alcohol recidivism, the following factors have been indicated in previous reports: abstinence period, presence of psychiatric comorbidity, poor compliance, family history of alcoholism, high-risk alcoholism relapse score (4-6)[36], poor social support, presence of young children, female sex, age < 50 years. An abstinence period before LT has been demonstrated as the predictive factor in most[11,19-21,31], but not all[22,24,32], publications.

Patient outcomes

The long-term survival rates of patients who underwent LT for ALD are reportedly 82%-92% at one year, and 72%-83% at 5 years[1,11,21,37,38]. These results are comparable to those of patients including all etiologies from different parts of the world (79%-83% at one year and 67%-77% at five years)[28,37,39]. Alcohol recidivism has been reported to impair long-term outcome[24,26,27,29-31], presumably due to its negative influence on the recipients, including alcohol toxicity, poor compliance, development of post-transplantation malignancies and occurrence of cardiovascular diseases. Rates of graft loss due to alcoholic recidivism range between 0% and 50%[21,27,30,40,41], and significant association of ALD patients with increased development of post-transplantation malignancy and occurrence of cardiovascular diseases were suggested[1,42].

Concerns on LT for acute alcoholic hepatitis without an abstinence period

Alcoholic hepatitis is a distinct clinical syndrome associated with recent or ongoing alcohol consumption, and its severity leads to high mortality exceeding 50%[35,43-46]. Medical treatment including the use of corticosteroids and/or pentoxifylline reduces the mortality rate to approximately 20%-30%[43,47]. Non-responsive patients suffer high mortality, and thus LT for alcohol hepatitis has been proposed in select patients[35,47,48]. However, alcoholic hepatitis is a controversial indication, or even a contraindication, for LT in most institutions[49,50] due to the high potential for alcohol recidivism, and conceivably due to the lack of pre-transplant abstinence period. A recent prospective multicenter study showed clear improvement on the odds of survival among patients unresponsive to medical therapy and followed with LT for severe alcoholic hepatitis[35]. The six-month and two-year survival rates among LT patients were significantly higher among

Table 1 Predictive factors for alcoholic recidivism

Ref.	Year	Alcoholic recidivism	Predictive factors
Gish *et al*[32]	2001	20%	Poor compliance and personality disorder
Jauhar *et al*[22]	2004	15%	Family history of alcoholism
DiMartini *et al*[19]	2006	42%	Alcohol dependence and an abstinence period
De Gottardi *et al*[11]	2007	12%	HRAR high score (4-6), presence of psychiatric comorbidity, and an abstinence period (≤ 6 mo)
Pfitzmann *et al*[21]	2007	19%	An abstinence period (< 6 mo), poor social support, presence of young children, and a poor psychosomatic prognosis
Tandon *et al*[31]	2009	24%	Pre-transplant abstinence
Karim *et al*[20]	2010	10%	An abstinence period (< 6 mo), female sex, presence of psychiatric comorbidity, age < 50 yr
Egawa *et al*[24]	2014	23%	Presence of psychiatric comorbidity

HRAR: High-risk alcoholism relapse.

non-LT patients (six months: 77% ± 8% *vs* 23% ± 8%, $P < 0.001$; two years: 71% ± 9% *vs* 23% ± 8%, $P < 0.001$). The survival rate of patients who underwent LT was comparable to that of patients who responded to medical therapy (77% ± 8% *vs* 85% ± 4%). The overall recidivism rate with relapse was 12%, with no case of alcoholic relapse within the initial six-month follow-up period after LT. Similar survival rates were reported in a retrospective study comparing LT outcomes for patients with alcoholic hepatitis to those with alcoholic cirrhosis (one year: 93% *vs* 88%; two years: 91% *vs* 84%; five years: 80% *vs* 78%)[48]. However, both studies mentioned an observable difference in society's readiness towards transplants for ALD and other self-inflicted liver diseases, despite their comparable mortality. In fact, criticism from the public is not present in response to LT for patients with fulminant hepatic failure stemming from voluntary acetaminophen poisoning, nor intravenous-drug users with acute hepatitis B virus infection[35,48]. In order to gain public acceptance, some sensitive issues surrounding LT for alcoholic hepatitis need to be addressed even though the medical benefits of LT have been proposed for strictly selected patients.

CONSIDERATIONS ON LDLT FOR ALD

Although there are many reports on DDLT for patients with ALD, there are few concerning LDLT. This is most likely because ALD is not a major primary disease for LT in the regions where LDLT is common, and DDLT is not practical due to the shortage of deceased donors. For instance, ALD accounts for only 2% of all primary transplantations in Japan, where 98% of LT has been performed through LDLT according to the registry by the Japanese Liver Transplantation Society[37]. Nevertheless, ALD is an important indication for LT following an annual increase of ALD recipients in Japan[37]. There are only two published reports on LDLT for ALD patients; one is a single-center study from our own institution[13], and the other is a multi-center questionnaire-based study in Japan[24].

Single-center study

Although the number of patients with ALD was limited in our single-center study, the results indicated a relatively low recidivism rate (8%) after LDLT for ALD patients selected based on a strict criteria that required the six-month rule, participation in Alcoholics Anonymous or equivalent rehabilitation program, consultation with a psychiatrist, and signed agreement declaring an intention of lifetime abstinence[13]. In addition, the study implied that pre-transplant abstinence was useful to observe possible recovery from liver failure as well as to identify patients who would not abstain from alcohol before and/or after LT. From this, we assumed that the role of abstinence before LDLT is to ensure positive effects on preventing post-transplant alcoholic recidivism even if results are not established and to recompense the potential risks the donor carried.

Multi-center study

In contrast, the rate of post-transplantation relapse in the multi-center study involving 38 institutions in Japan, with selection criteria for ALD patients determined at each institution, ranged from 7% to 95%[24]. The study noted the possibility that relatives who donated their organs, notwithstanding operation risks, may have allowed recipients' alcohol consumption after LT. In fact, recidivism rates of patients whose parents or siblings were donors ranged from 28% to 50%, slightly higher than those whose donors were spouses (13%) or relatives (23%). Considering the relatively high alcoholic relapse rate after LDLT, the study suggested that DDLT may be more suitable for patients with ALD.

Patient outcomes

The long-term survival rate of patients who underwent LDLT for ALD was comparable with that of DDLT[1,11,21,37,38]; 100% at one year and 91% at five years in the single-center study[13], and 81% at one year and 76% at five years from data in the registry of the Japanese Liver Transplantation Society[37]. Similar to DDLT[21,26,27,29,30], the long-term survival rate for relapsing patients was significantly lower than that for abstinent patients (one year: 100% *vs* 100%; three years: 95% *vs* 99%; five years: 90% *vs* 96%, $P = 0.01$)[24].

Public and ethical perspectives on LDLT for ALD

LDLT for ALD may seem to be generally accepted by

society from a public point of view because, unlike with DDLT, it does not conflict with organ allocation issues. Nevertheless, ethical issues remain. First, liver transplantation professionals are confronted with difficult situations caused by the dilemma between strong willingness displayed by the family to donate and compliance with pre-transplant abstinence rule. For instance, professionals working in most institutions feel obliged to inform patients who may have prospective living donors and their family members that the requirement for a six-month abstinence period is still applicable, even when some of the patients are not expected to survive more than six months. Second, recidivism is not readily accepted by society even if the organ is donated by a family member because LT is supported by national- and/or social- welfare systems in general. LDLT for ALD, inseparable from the public opinion, becomes a complicated topic that requires a viewpoint slightly different from DDLT for ALD when addressing their issues.

The extremely limited number of reports on LDLT for ALD led to difficulty in achieving consensus on optimal selection criteria for ALD patients as well as on strategies for preventing alcoholic recidivism after LT. To improve current status of LDLT for ALD and support liver transplantation professionals involved in the treatment for ALD, a significant increase in the number of reports on this topic are essential, not to mention a well-designed prospective study.

CONCLUSION

Alcoholic liver disease remains a commonly recognized indication for LT in Europe and the United States, with an increasing presence in Asia as well. ALD is a self-inflicted disease in which patients may possibly relapse to alcohol consumption after transplantation. These facts still raise questions on sharing organs as a public resource for DDLT. LDLT, unlike DDLT, may not necessarily link to organ allocation issues, but it is nonetheless inseparable from the public eye as an ethical standpoint. Considerable efforts to improve post-transplant outcome are required to recompense the potential risks to living donors.

Prevention of alcoholic recidivism is regarded as the most important post-transplant treatment because alcohol impairs long-term outcome of ALD patients. Although not conclusive, an abstinence period and presence of psychiatric comorbidity are potential predictive factors for post-transplantation recidivism. Organ donations from relatives do not suppress alcoholic recidivism as the recipient's alcohol consumption tends to be tolerated by the donors themselves. Incidentally, recent studies promote the medical benefits of LT for patients with alcoholic hepatitis whose medical therapy was ineffective, but recidivism is anticipated in these patients who likely continue to consume large volumes of alcohol. LT for alcoholic hepatitis is still a highly controversial issue from the public point of view, and needs to be resolved.

Well-designed prospective studies on DDLT/LDLT are essential to resolve the debatable issues on LT for ALD. Establishment of accurate predictive factors for alcoholic recidivism, benefits and optimal duration of pre-transplant abstinence, and appropriate indication criteria of LT for ALD are among high priority issues. Further evaluations on these issues will help to more effectively control alcoholic recidivism and improve, not only the outcome of LT for ALD patients, but also acceptance from society.

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CASE CONTROL STUDY

Circulating microRNA, miR-122 and miR-221 signature in Egyptian patients with chronic hepatitis C related hepatocellular carcinoma

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Abstract

AIM: To explore the potential usefulness of serum miR-122 and miR-221 as non-invasive diagnostic markers of hepatitis C virus (HCV)-related hepatocellular carcinoma (HCC).

METHODS: This prospective study was conducted on 90 adult patients of both sex with HCV-related chronic liver disease and chronic hepatitis C related HCC. In addition to the 10 healthy control individuals, patients were stratified into; interferon-naïve chronic hepatitis C (CH) (n = 30), post-hepatitis C compensated cirrhosis (LC) (n = 30) and treatment-naïve HCC (n = 30). All patients and controls underwent full clinical assessment and laboratory investigations in addition to the evaluation of the level of serum miRNA expression by RT-PCR.

RESULTS: There was a significant fold change in serum miRNA expression in the different patient groups when compared to normal controls; miR-122 showed significant fold increasing in both CH and HCC and significant fold decrease in LC. On the other hand, miR-221 showed significant fold elevation in both CH and LC groups and significant fold decrease in HCC group (P = 0.01). Comparing fold changes in miRNAs in HCC group *vs* non HCC group (CH and Cirrhosis), there was non-significant fold elevation in miR-122 (P = 0.21) and significant fold decreasing in miR-221 in HCC *vs* non-HCC (P = 0.03). ROC curve analysis for miR-221 yielded 87% sensitivity and 40% specificity for the differentiation of HCC patients from non-HCC at a cutoff 1.82.

CONCLUSION: Serum miR-221 has a strong potential to serve as one of the novel non-invasive biomarkers of HCC.

Key words: MiRNA; Hepatocellular carcinoma; Serum

Core tip: In the current study a signature of circulating miRNAs (miR-122 and miR-221) was evaluated. miR-221 was differentially expressed between patients with hepatocellular carcinoma and those without (chronic hepatitis and liver cirrhosis) with lower serum level of miR-221 in former group of patients in comparison to later one. miR-221 yielded 87% sensitivity and 40% specificity in differentiating between both groups at a cutoff 1.82 folds. The present study emphasizes that circulating miR-221 deserves further attention as a potential non-invasive biomarkers for hepatocellular

carcinoma.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary liver cancer, and the fifth most common cancer worldwide[1]. The last decade has witnessed a significant rise in the incidence of HCC[2-4] with a specially high incidence reported in Egypt[5]. A direct role of hepatitis C virus (HCV) in hepatocarcinogenesis has been suggested[6]. However, it seems that cirrhosis is the common route through which several risk factors act and induce carcinogenesis[7].

Currently available blood tumor markers are far from optimal. Alfa-fetoprotein (AFP), Lens culinaris agglutinin-reactive AFP (AFP-L3) and des-carboxyprothrombin (DCP) perform poorly in the surveillance mode and early detection of HCC[8]. The Practice Guidelines of the American Association for the Study of Liver Diseases (AASLD) (July 2010) rejected AFP whether for the surveillance or the diagnosis of HCC. This highlights the need for other methods that would be minimally-invasive, simple and reliable for the early detection of HCC.

MicroRNA (miRNA) is a non-coding *RNA* gene product that negatively controls gene expression by altering the stability or translational efficiency of its target mRNAs[2]. MiRNAs regulate several biological processes, such as cell differentiation, apoptosis and proliferation. miRNAs have been reported to be aberrantly present in cancers whether through up- or down-regulation in neoplastic cells compared with their normal counterparts[3,4]. What makes miRNAs even more interesting is that several recent studies have demonstrated that miRNAs are detectable and stable in plasma and serum[4-6].

The goal of the present study was to evaluate circulating serum miRNAs (miR-122 and miR-221) expression levels in Egyptian patients with HCC as well as in patients with HCV-related chronic liver disease to explore their potential as novel non-invasive markers for diagnosis of HCV-related HCC.

MATERIALS AND METHODS

During the period between March and June 2012 serum samples were collected from consecutive HCV-infected patients presenting to our outpatient department: 30 with chronic HCV alone (CH), 30 with HCV-related cirrhosis (LC) and 30 with HCV-related HCC. Serum samples were also collected from 10 age and gender-matched healthy volunteers (defined as those with normal transaminases, normal hepatic ultrasound and negative for HBsAg, HBc-Ab and HCV RNA-PCR). All patients were recruited after a written informed consent and the study protocol was approved by the ethics review committee of Cairo University hospital. Exclusion criteria included: patients with chronic HBV infection or any other identifiable cause for chronic hepatitis other than HCV, previous treatment for HCC or antiviral therapy for HCV and any associated malignancies other than HCC.

RNA extraction

For the real-time PCR RNAs were extracted from serum using TRIzol according to the manufacturer's instruction. The RNA purity was assessed by the RNA concentration and quantified by NanoDrop ND-1000 (Nanodrop, United States). Single-stranded cDNAs were generated using the RT kit (Qiagen, Valencia, CA, United States) according to the manufacturer's directions (miSCript miRNA PCR system, miRneasy mini kit for miRna extraction, miScript RT Ⅱ for miRna reverse transcription, miSCript Primer Assay and miSCript SYBR Green PCR Kit for PCR amplification.

RNA quantification

PCR quantification experiments were performed with PCR (Applied Biosystems; Foster City, CA) using the SYBR Green PCR Master Mix according to the manufacturer's protocol. The primers for microRNA-122, -221 and housekeeping gene were supplied by Qiagene, Germany (catalog numbers 3416, 3857 and 33712). The housekeeping miRNA SNORD68 was used as the endogenous control. Fluorescence measurements were made in every cycle and the cycling conditions used were: 95 ℃ for 30 s, and 40 cycles of 95 ℃ for 5 s and 60 ℃ for 34 s.

Expression of miRNAs was reported as ΔCt value. The ΔCt was calculated by subtracting the Ct values of miRNA SNORD68 from the Ct values of the target miRNAs. As there is an inverse correlation between ΔCt and miRNA expression level, lower ΔCt values were associated with increased miRNA. The resulting normalized ΔCt values were used in calculating relative expression values by using 2- Δ(Ct), these values are directly related to the miRNA expression levels. The 2-ΔΔ (Ct) method was used to determine relative-quantitative levels of individual miRNAs.

Statistical analysis

Patients were categorized into 4 groups; normal, CH, Cirrhosis and HCC. Further comparisons were performed between HCC group and Non-HCC (CH and cirrhosis). Quantitative variables were expressed by mean ± SD or expressed by median and inter quartile range (IQR) for non-parametric data. They were compared by *t*-student or ANOVA test when appropriate. Qualitative variables were compared by χ^2 or Fischer's exact test when appropriate. AFP levels were transformed into their log values

Table 1 Demographic parameters of patients

	HCC (n = 30)	Cirrhosis (n = 30)	CH (n = 30)	Normal (n = 10)	P value
Age (yr) Mean ± SD	60.27 ± 8.20^{C}	55.07 ± 7.35^{B}	38.20 ± 8.21^{A}	40.89 ± 16.85^{A}	⩽ 0.001
Gender (male)	25 (83.3)	21 (70)	22 (73.3)	6 (66.7)	0.6
Hb (g/dL)	11.44 ± 2.85^{B}	10.53 ± 2.00^{B}	14.23 ± 1.58^{A}	14.03 ± 2.48^{A}	< 0.001
WBC × 10^3/mm^3	5.73 ± 2.56^{A}	7.08 ± 3.77^{A}	6.15 ± 2.13^{A}	7.38 ± 2.72^{A}	0.217
Platelets 10/mm^{3D}	126.00 ± 74.05^{B}	116.10 ± 68.94^{B}	228.80 ± 59.74^{A}	271.3 ± 116.7^{A}	< 0.001
Total bilirubin (0.1-1.2 mg/dL)	1.88 ± 2.01^{A}	4.29 ± 6.75^{B}	0.74 ± 0.26^{A}	0.79 ± 0.36^{A}	< 0.001
ALT (0-42 IU/L)	66.59 ± 44.59^{B}	32.15 ± 23.59^{A}	66.78 ± 36.56^{B}	29.16 ± 19.96^{A}	< 0.001
AST (0-42 IU/L)	119.99 ± 56.12^{B}	63.81 ± 39.49^{A}	59.67 ± 45.04^{A}	45.64 ± 54.32^{A}	< 0.001
ALP (0-290 IU/L)^{D}	394.8 ± 282.28^{A}	304.89 ± 191.85^{A}	203.58 ± 82.37^{AB}	198.8 ± 139.1^{B}	0.003
Albumin (3.5-5.5 g/dL)^{D}	3.16 ± 0.40^{C}	2.49 ± 0.54^{B}	4.22 ± 0.36^{A}	4.09 ± 0.92^{A}	< 0.001
PC %	69.63 ± 16.25^{C}	51.58 ± 17.12^{B}	88.24 ± 10.89^{A}	97.63 ± 11.77^{A}	< 0.001
AFP log10 ng/dL	2.50 ± 1.19^{B}	0.79 ± 0.54^{A}	0.59 ± 0.38^{A}	NA	< 0.001

^{A,B,C,D}Groups with different letters show significant difference, those with similar letters show no significant difference. HCC: Hepatocellular carcinoma; NA: Not applicable. CH: Chronic hepatitis C.

Table 2 Tumor-related characteristics (n = 30)

	Parameter	Number (%)
AFP level (0-10)	Normal	4 (13.4%)
	Elevated	26 (86.6%)
PS	PS 0	24 (80)
	PS 1-2	4 (13.4)
	PS > 2	2 (6)
BCLC	Stage 0	0 (0)
	Stage A	1 (3.8)
	Stage B	19 (73.1)
	Stage C	4 (15.4)
	Stage D	2 (7.7)
Number of focal lesions	Single	17 (56.7)
	Multiple	13 (43.4)
Site of focal lesions	Right lobe	18 (60)
	Left lobe	5 (16.7)
	Both	7 (23.3)
Tumor size by CT	< 3 cm	1 (3.3)
	3-5 cm	12 (40)
	> 5 cm	17 (56.7)
Portal vein invasion	Yes	7 (23.3)
	No	23 (76.7)

PS: Performance status; AFP: Alfa-fetoprotein; CT: Computed tomography.

to undergo parametric statistical tests. Receiver operator characteristic (ROC) curves were constructed to assess the value of miRNA in diagnosing HCC and to assess area under the curve (AUROC). AUROC less than 0.60 with P value > 0.05 is considered unreliable for ROC curve. Spearmen and Pearson correlations were done for correlating quantitative variables. In all tests, P value was considered significant if less than 0.05.

RESULTS

This study was conducted on 100 participants stratified into: Group1: thirty patients with HCV-related HCC who were diagnosed according to EASL guidelines 2012; Group2: thirty patients with hepatitis C related liver cirrhosis; Group 3: thirty non-cirrhotic patients with chronic hepatitis C viral infection (CH), while Group 4 included ten age and gender-matched healthy volunteers (defined as those with normal hepatic ultrasound and transaminases and negative for hepatitis B and C by PCR) considered as internal reference.

The demographic and pathologic features of the studied participants are shown in Tables 1 and 2**.** There was a significant difference between the diseased groups regarding age (P < 0.001). Regarding gender difference; males were predominant in HCV related liver disease patients in the three groups and they represented 83.3%, 70%, 73.3% in HCC, cirrhosis and CH groups respectively with no statistically significant difference between the studied groups (P = 0.60).

miR-122 serum levels

Analysis of median fold change in expression level of miR-122 in patients' sera in comparison to the normal control group showed that miR-122 displayed significant fold decrease in expression in cirrhosis group (0.8) and significant fold increasing in expression level in both CH (2.1) and HCC (2.15) groups (P ⩽ 0.01), Table 3. Comparing serum miR-122 expression level between different studied groups displayed an increasing tendency towards statistical significant fold elevation in expression of miR-122 in serum of HCC patients (2.15) in comparison to liver cirrhosis (0.8) with P value 0.083 (AUC = 0.646), Figure 1. No significant fold change in miR-122 expression was found between either (HCC *vs* CH groups) or (CH *vs* cirrhosis groups). MiRNA122 showed non-significant up-regulation in HCC patients in comparison to non-HCC patients (CH and Cirrhosis); (P = 0.21).

miR-221 serum levels

Analysis of fold change in expression level of miR-221 in patients' sera in comparison to the normal control group showed significant fold decrease in HCC group and significant fold increase in expression level in CH and cirrhosis groups in comparison to normal control group (< 0.01), Table 4. There was a statistically significant fold decreasing in serum miR-221 levels of HCC patients (0.92) in comparison to cirrhosis (3.4) and in comparison to CH (1.7) (P = 0.05 and 0.06 respectively). On the other

Table 3 MiR-122 serum levels in the different groups

Fold of change comparing to normal				
Group	**HCC *n* = 30**	**Cirrhosis *n* = 30**	**CH *n* = 30**	***P* value**
miR-122 (IQR)	2.15 (7.3)	0.8 (3.7)	2.1 (9)	< 0.01 0.083[1] 0.572[2] 0.417[3]
Group	HCC *n* = 30	Non HCC (CH + cirrhosis) *n* = 60		*P* value
miR-122 (IQR)	2.15 (7.3)	1.75 (6.8)		0.21 (NS)

[1]HCC *vs* cirrhosis; [2]HCC *vs* CH; [3]Cirrhosis *vs* CH. HCC: Hepatocellular carcinoma; CH: Chronic hepatitis C; IQR: Inter quartile range; NS: Non significant; PVP: Positive predictive value; NVP: Negative predictive value.

Table 4 MiR-221 serum levels

Fold of change in comparison to normal				
	HCC *n* = 30	**Cirrhosis *n* = 30**	**CH *n* = 30**	***P* value**
miR-221 (IQR)	0.92 (0.88)	3.4 (19.2)	1.7 (2.6)	> 0.01 0.05[1] 0.06[2] 0.214[3]
	HCC 0.92 (0.88)	Non HCC 1.81 (7.75)		0.03 (S)

[1]HCC *vs* LC; [2]HCC *vs* CH; [3]LC *vs* CH. HCC: Hepatocellular carcinoma; CH: Chronic hepatitis C; IQR: Inter quartile range; LC: Liver cirrhosis.

hand, there was no statistical significant fold change in serum miR-221 expression level between (CH *vs* cirrhosis groups) ($P = 0.214$).

miRNA-221 displayed significant fold decrease in HCC group (0.92) compared to non-HCC patients (CH and cirrhosis) (1.81) (*P* value 0.03). At a cut-off of 1.82 folds, miR-221 yielded 87% sensitivity and 40% specificity in differentiating between both groups. Figure 2, Table 5.

Correlation of miRNA's with features of HCC

There was no statistically significant correlation between serum expression level of studied miRNAs and serum AFP level in the different studied groups of patients**.** No significant correlation was found between the two miRNAs and tumor size, Child-pugh grade in HCC group of patients.

Correlation of miRNA's with severity of hepatic dysfunction

Serum miRNA-122 expression level showed statistically significant correlation with serum necro-inflammatory markers of the liver [aspartate transaminase (AST) and alanine transaminase (ALT) levels) in CH group (*P* value 0.034 and 0.030 respectively), Table 1.

DISCUSSION

Over the last 2 decades it has become common practice

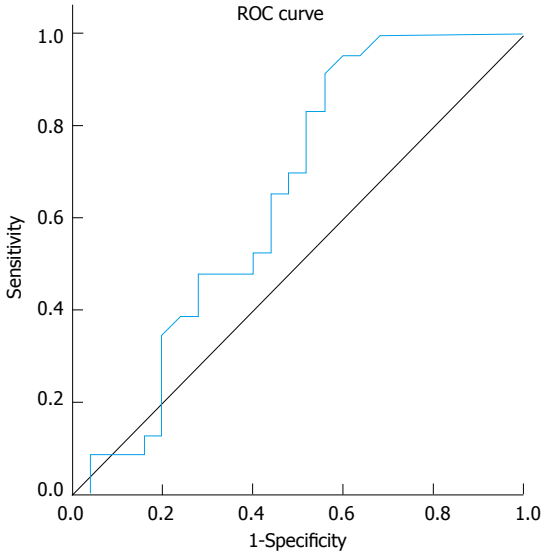

Figure 1 Receiver operator characteristic curve for miR-122 as a discriminant of hepatocellular carcinoma *vs* cirrhosis patients.

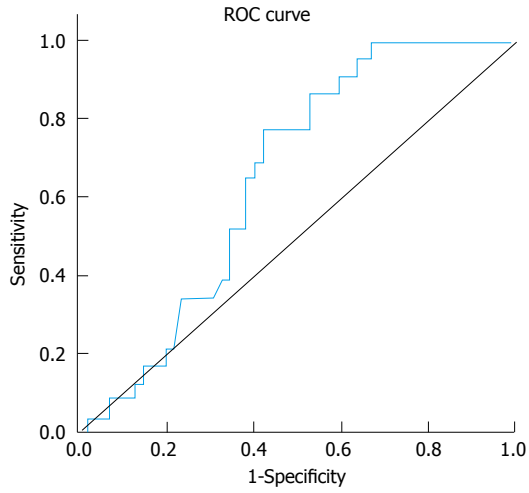

Figure 2 Receiver operator characteristic curve for miR-221 as a discriminant between hepatocellular carcinoma *vs* non-hepatocellular carcinoma.

to use tumour markers, mainly AFP, for the screening of HCC. However, performance of tumor markers has not been optimal with the sensitivity and specificity of AFP and PIVKA-II in the range of 39%-64% and 76%-91%, and 41%-77% and 72%-98%, respectively[9,10], the quest for an optimal tumor marker hence continues. miRNAs have been implicated in roles affecting cellular proliferation and oncogenesis[11]. Cellular miRNAs have been linked with HCC[12]. Their availability in the circulation makes them a tempting target for early tumor detection[12]. The aim of the present study was to explore the potential usefulness of serum miR-122 and miR-221 as novel noninvasive markers for diagnosis of HCV related hepatocellular carcinoma in Egyptian patients.

Most of our HCC patients were within Child-Pugh A and B classifications (56.7%, 36.7% respectively), 73.1% were stage B on BCLC scoring system[13]. This could be explained by the fact that most of them were recruited

Table 5 Diagnostic performance of miR-221 for discriminating patients with hepatocellular carcinoma from those without

AUC	*P* value	Best cutoff	Sensitivity	Specificity	PPV	NPV
0.655	0.03	< 1.82	0.87	0.40	0.47	0.83

HCC: Hepatocellular carcinoma; PVP: Positive predictive value; NVP: Negative predictive value.

while being assessed for the possibility of interventional treatment. Another possible explanation for rather good liver condition seen in HCC series could be attributed to that with implementing surveillance programs, allowing detecting tumors at an early stage in well compensated patients. Alfa fetoprotein level was normal (< 10 ng/dL) in 13.4% of recruited HCC patients. Similar finding was observed by Tateishi *et al*[14] who suggested that not all tumors secrete AFP, and serum levels are normal in up to 40% of small HCCs. It was also showed that α-Fetoprotein alone is not recommended for the diagnosis of HCC and studies showed that its cut off value should be set at 200 ng/mL.

Analysis of fold changes in expression level of miR-122 displayed significant fold increase in expression level in chronic hepatitis C group (2.1) and significant fold decrease in expression in cirrhotics (0.8) in comparison to normal controls. miR-122 is present abundantly in hepatocytes with much lower levels in the circulation in healthy subjects. With hepatocyte injury miR-122 is released in the circulation more readily and serum levels rise. With the eventual loss of hepatocytes and development of fibrosis with proliferation of myelofibroblasts and accumulation of extracellular matrix the circulating miR-122 levels drop again[15].

In the current study there was significant fold rise in serum expression level of miR-122 in HCC group in comparison to normal control group (*P* value < 0.01). Matching our results, Trebicka *et al*[15] who studied hepatic miR-122 expression in 43 HCV related HCC in comparison to 3 healthy liver samples using qRT-PCR; miR-122 was strongly up-regulated in malignant liver nodules in comparison to healthy liver. They suggested that miR-122 might down regulate target mRNA of unknown tumor suppressor genes and thus lead to further tumor growth[15].

In a study on hepatitis B patients Xu *et al*[16] suggested that cancer-induced hepatocyte damage would release the abundant intracellular miR-122 into the circulation, the stability of miRNA would be reflected by easily detectable high blood levels[17]. In contrast to our results, significant down regulation of miR-122 in HCC compared to normal liver tissue was reported by Meng *et al*[18], Wang *et al*[19] and Huang *et al*[20] who compared miR-122 expression profile of 3 different pairs of tumor and normal human liver-derived RNA and 20 HCC liver tissues (mixed etiologies) to normal tissues respectively using microarray[18,19,20]. Similarly a significant down regulation in miR-122 in 19 HBV related HCC liver tissue in comparison to paired healthy liver by next-generation sequencing was reported by Connolly *et al*[21].

Ladeiro *et al*[22] have established significant down expression of miR-122 in 28 HCC liver tissues (mixed etiologies) in comparison to 4 healthy liver tissues by qRT-PCR.

In our series, no statistically significant correlation could be verified between serum miR122 expression level and patient characters (age), liver synthetic functions tests (Albumin, bilirubin and PC), or serum AFP level in HCC *vs* non HCC group (CH and cirrhosis). However, in the chronic hepatitis groups serum miR-122 was correlated with higher AST and ALT levels, further solidifying the theory regarding the initial rise in miR-122 levels due to hepatocyte inflammation and destruction followed by a drop in the levels with the developing fibrosis. Köberle *et al*[23] also reported significant correlation between serum miR-122 expression level and necro-inflammatory markers (AST, ALT), and Albumin but no significant correlation was found with bilirubin in HCC patients[23].

Perhaps the most significant finding in our study was related to miR-221. Analysis of fold change in expression level of miR-221 in patients' sera of HCV associated liver disease (CH and cirrhosis) in comparison to normal control group showed significant fold increase in expression level in CH and cirrhosis groups in comparison to normal control group (< 0.01). Also a significant fold decrease in serum miR-221 in HCC group (0.92) in comparison to normal control was noticed. We assumed that with the progression of liver disease from chronic hepatitis to cirrhosis the increased activity of hepatic stellate cells was associated with increase miR-221 expression level, such high level stimulated tumorigenesis and increase level of miR-221 in tissue, but as miR-221 is anti apoptotic so serum miR-221 didn't show similar increase. In contrast to our results many studies established up regulation of miR-221 in HCC in relation to normal control, *e.g.*,[18,19,20,24]. However, most these studies assessed tissue miR-221 rather than serum levels .The different results could also be explained by technical variations including sampling methods and freezing and RNA isolation procedures. The etiology of liver disease is also variable in different studies including viral and alcoholic. The stage of the disease is also a source of variation especially that it is still not evident how miRNA expression changes with fibrosis progression. Different studies have also used different control samples for normalization, *e.g.*, non-HCC tissue from the same patient, healthy liver tissue from another subject or patients with the same pathology but not HCC, this is especialy relevant to studies assessing tissue miRNA levels[25].

Similar to what was previously reported by Rong *et al*[26], we found no statistically significant correlation could be verified between serum miR-221 expression level and patient characters (age), laboratory values (AST, ALT), liver synthetic functions tests (Albumin, bilirubin and PC), or serum a-fetoprotein level in HCC *vs* non HCC group (CH and cirrhosis) and no statistically significant correlation could be found between the clinic-pathological parameters of hepatic focal lesion, *e.g.*, (number of focal lesions, Child

score, biggest diameter of focal lesion BCLC, and portal vein invasion) and miR-221 expression level ($P \geqslant 0.05$)[26].

Circulating miR-221 level is significantly up-regulated in the serum of HCV infected patients. It has some value in the differentiation between HCV patients with hepatocellular carcinoma and those without with 87% sensitivity and 40% specificity. It may be able to serve as a promising non-invasive diagnostic marker for HCC. Better results could be obtained if combined with other markers and testing a panel of miRNA's collectively could ultimately serve as a reliable diagnostic test for HCC.

COMMENTS

Background

Hepatocellular carcinoma (HCC), the most common type of liver cancer, is amongst the top three leading causes of cancer-related deaths worldwide with a median survival of only six to eight months. This poor outcome is related to the late detection, with more than two thirds of patients diagnosed at advanced stages of disease. Thus, surveillance of populations at-risk may detect tumors at an early stage when curative interventions can be implemented. The performance of available circulating biomarkers in the screening and diagnostic settings of HCC is sub-optimal.

Research frontiers

MiRNAs constitute a large class of genes that encode short RNAs (19-24 nucleotides long), which play key roles in development and differentiation, by the post-transcriptional regulation of protein coding genes. At present, miRNAs have a widely recognized role in human carcinogenesis, including hepatocarcinogenesis, and many experimental evidences indicate that they may act as oncogenes or tumor suppressor genes regulating the expression of crucial protein coding genes. MiRNAs have been proposed as possible novel biomarkers for cancer diagnosis.

Innovations and breakthroughs

In the current study a signature of circulating miRNAs (miR-122 and miR-221) was evaluated. MiR-221 was differentially expressed between patients with HCC and those without (chronic hepatitis and cirrhosis) with lower serum level of miR-221 in former group of patients in comparison to later one. MiR-221 yielded 87% sensitivity and 40% specificity in differentiating between both groups at a cutoff 1.82 folds.

Applications

The present study emphasis that circulating miR-221 deserves much attention as potential non invasive biomarkers for HCC in the diagnostic setting.

Terminology

HCC: Hepatocellular carcinoma; Non HCC: Chronic hepatis C group of patients and patients with liver cirrhosis.

Peer review

The manuscript entitled "Circulating microRNA, miR-122 and miR221 Signature in Egyptian Patients with Chronic Hepatitis C Related Hepatocellular Carcinoma". The manuscript is interesting.

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CASE REPORT

Hemodynamic effects of ambrisentan-tadalafil combination therapy on progressive portopulmonary hypertension

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Abstract

Intravenous epoprostenol is recommended for World Health Organization functional class (WHO-FC) Ⅳ patients with pulmonary arterial hypertension (PAH) in the latest guidelines. However, in portopulmonary hypertension (PoPH) patients, advanced liver dysfunction and/or thrombocytopenia often makes the use of intravenous epoprostenol challenging. Here we report the cases of two WHO-FC Ⅳ PoPH patients who were successfully treated with a combination of two oral vasodilators used to treat PAH: ambrisentan and tadalafil. Oral vasodilator therapy using a combination of ambrisentan and tadalafil may be a safe and effective therapeutic option for WHO-FC Ⅳ PoPH patients and should be considered for selected patients with severe and rapidly progressing PoPH.

Key words: Portopulmonary hypertension; Ambrisentan; Tadalafil; Thrombocytopenia

Core tip: Advanced liver dysfunction and/or thrombocytopenia often hamper the use of intravenous epoprostenol in patients with portopulmonary hypertension (PoPH). However, recent progress in the oral treatment for pulmonary hypertension (PH) has enabled better clinical outcome in severe PH patients. Here we report two World Health Organization functional class IV patients with PoPH and thrombocytopenia who were successfully treated with ambrisentan and tadalafil. Oral vasodilator therapy using a combination of ambrisentan and tadalafil may be a safe and effective therapeutic option for patients with PoPH and advanced thrombocytopenia.

Yamashita Y, Tsujino I, Sato T, Yamada A, Watanabe T, Ohira H, Nishimura M. Hemodynamic effects of ambrisentan-tadalafil combination therapy on progressive portopulmonary hypertension. *World J Hepatol* 2014; 6(11): 825-829 Available from: URL: http://www.wjgnet.com/1948-5182/full/v6/i11/825.htm DOI: http://dx.doi.org/10.4254/wjh.v6.i11.825

INTRODUCTION

Reportedly, 2%-6% of patients with portal hypertension also develop pulmonary hypertension (PH); this combined disorder is called portopulmonary hypertension (PoPH)[1-3]. According to the latest guidelines[4], PoPH is classified in the pulmonary arterial hypertension (PAH) spectrum. It is recommended that PoPH patients be managed similarly to those with other forms of PAH, while considering the presence of liver disease and its consequences[5]. Although intravenous epoprostenol treatment is recommended for World Health Organization functional class (WHO-FC) Ⅳ PAH patients, advanced liver dysfunction and/or thrombocytopenia

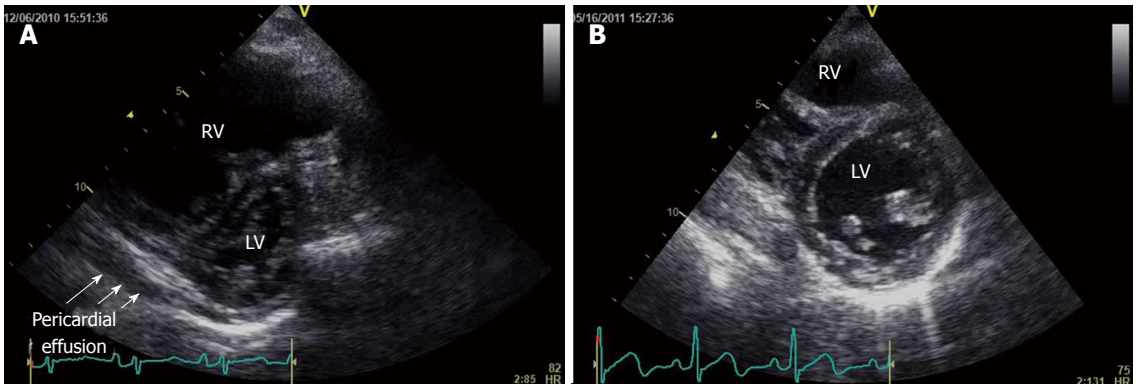

Figure 1 Transthoracic echocardiography performed before and after treatment. A: Transthoracic echocardiography (TTE) performed before treatment. The parasternal short axis view at the basal level in diastole shows pronounced interventricular septal deviation toward the left ventricle accompanied by pericardial effusion; B: TTE performed after treatment. The parasternal short axis view at the basal level in diastole shows a decrease in the interventricular septal deviation toward the left ventricle. Pericardial effusion has disappeared. RV: Right ventricle; LV: Left ventricle.

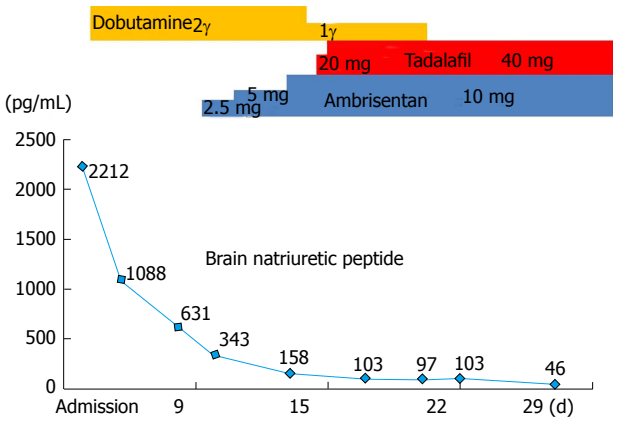

Figure 2 Clinical course and treatment of case 1. Plasma brain natriuretic peptide concentration remarkably decreased from 2212 pg/mL to 46 pg/mL by intravenous dobutamin infusion and subsequent oral ambrisentan-tadalafil combination treatment.

often make such invasive management difficult in PoPH patients. Here we describe two WHO-FC Ⅳ PoPH patients who favorably responded to the combined use of ambrisentan and tadalafil, oral vasodilators that have been proven to be effective against PAH.

CASE REPORT

Case 1

In September 2010, a 56-year-old man with hepatitis B virus-related cirrhosis, esophageal varix, and hepatocellular carcinoma was referred to our department with abnormal findings on electrocardiogram. Doppler echocardiography indicated increased systolic right ventricular pressure, estimated by a tricuspid regurgitation pressure gradient (TRPG) of 79 mmHg. Right heart catheterization (RHC) also revealed an increased mean pulmonary artery pressure (PAP) of 40 mmHg and an increased pulmonary vascular resistance (PVR) of 6.38 Wood units. PoPH was diagnosed; however, his clinical course (WHO-FC Ⅰ) was modest. Accordingly, we decided to employ a careful wait-and-watch approach.

However, 3 mo later, the patient experienced rapid progression of exertional dyspnea, facial edema, and syncope, and he was consequently admitted to our department. He presented with jugular venous distention, pretibial pitting edema, and a pronounced pulmonary component of the second heart sound. Laboratory data revealed advanced thrombocytopenia (5.0×10^4/mL), an increased D-dimer level (9.38 μg/dL), an increased indirect bilirubin level (4.9 mg/dL), a mildly increased transaminase level (aspartate aminotransferase, 69 U/L; alanine aminotransferase, 40 U/L), and an increased plasma brain natriuretic peptide (BNP) level (2212 pg/mL). Transthoracic echocardiography revealed right ventricular dilatation and severe interventricular septal deviation toward the left ventricle, accompanied by pericardial effusion (Figure 1A). The pulmonary artery systolic pressure, estimated on the basis of TRPG, was 120 mmHg. Abdominal computed tomography (CT) revealed dilatation of the splenic, esophageal, and umbilical veins, suggestive of portal hypertension, whereas ventilation/perfusion lung scintigraphy revealed no significant mismatch. These results indicated rapid progression of PoPH; therefore, dobutamine (2 μg/kg per minute) was initiated. RHC revealed that the mean PAP was increased to 55 mmHg, cardiac index (CI) was decreased to 2.49 L/min per square, and PVR was increased to 10.9 Wood units.

Because of comorbid advanced thrombocytopenia and hepatocellular carcinoma, we initiated ambrisentan at a dose of 2.5 mg once daily, added tadalafil at a dose of 20 mg once daily, and subsequently increased the dose of the two agents in turn to reach the maximum dose (ambrisentan, 10 mg/d; tadalafil, 40 mg/d) in 9 d. After a month, the patient's heart failure symptoms and signs had improved and his plasma BNP level had decreased (Figure 2).

At a follow-up assessment 5 mo later, the patient's WHO-FC had improved from Ⅳ to Ⅱ and his plasma BNP level had decreased to 44.4 pg/mL. Echocardiography revealed a decrease in TRPG from 95 to 56.2 mmHg, a less pronounced interventricular septal shift toward the left ventricle, and no evidence of pericardial effusion (Figure 1B). Repeat RHC revealed a decrease in

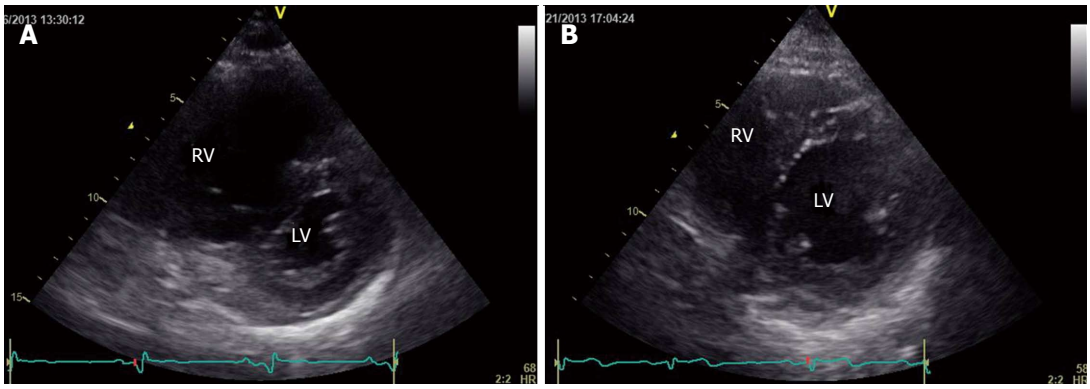

Figure 3 Transthoracic echocardiography performed before and after treatment. A: Pre-treament. Transthoracic echocardiography (TTE) performed before treatment. The parasternal short axis view at the basal level in diastole shows pronounced interventricular septal deviation toward the left ventricle accompanied by pericardial effusion; B: Five-month after treament. TTE performed after treatment. The parasternal short axis view at the basal level in diastole shows a decrease in the interventricular septal deviation toward the left ventricle. The pericardial effusion has disappeared. RV: Right ventricle; LV: Left ventricle.

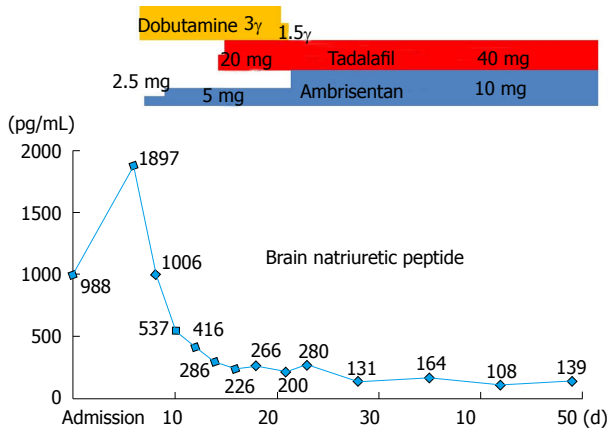

Figure 4 Clinical course and treatment of case 2. Plasma brain natriuretic peptide concentration remarkably decreased from 1897 pg/mL to 139 pg/mL by intravenous dobutamin infusion and oral ambrisentan-tadalafil combination treatment.

mean PAP (34 mmHg) and PVR (6.4 Wood units) and improvement in CI (4.27 L/min per square).

Case 2

In April 2013, a 70-year-old man was referred to our department with rapid worsening of exertional dyspnea (WHO-FC Ⅳ) and suspected PH on echocardiography. He had been diagnosed with liver cirrhosis from excessive alcohol consumption, hepatocellular carcinoma, and esophageal varix approximately 9 years previously. He presented with jugular venous distention, hepatomegaly, and splenomegaly as well as a pronounced pulmonary component of the second heart sound and a third heart sound on chest auscultation. Laboratory data revealed advanced thrombocytopenia (3.5×10^4/mL), an increased D-dimer level (4.39 μg/dL), mild hepatic dysfunction (Child-Pugh B), and an increased plasma BNP level (988 pg/mL). Echocardiography revealed right ventricular dilatation and severe interventricular septal deviation toward the left ventricle accompanied by pericardial effusion (Figure 3A). Systolic PAP was 125 mmHg, as estimated by TRPG. CT suggested dilation of the esophageal vein. Ventilation/perfusion lung scintigraphy revealed a mismatch in the left upper lobe, although it was limited and not compatible with chronic thromboembolic PH. Cardiac magnetic resonance imaging indicated a dilated right ventricle and decreased right ventricular ejection fraction (RVEF; 25.8%). A hemodynamic study with oxygen at 5 L/min revealed an increased mean PAP (62 mmHg), a decreased CI (1.43 L/min per square), and an increased PVR (18.5 Wood units).

Based on findings indicative of portal hypertension and RHC findings, PoPH was diagnosed. We initiated dobutamine at 2 μg/kg per minute to support cardiac function. We then initiated ambrisentan at a dose of 2.5 mg once daily. and added tadalafil at a dose of 20 mg once daily. The doses of these two agents were then increased in turn to reach the maximum dose (ambrisentan, 10 mg/d; tadalafil, 40 mg/d) in 15 d.

Within a month and a half, the patient's plasma BNP level had decreased, right heart failure signs had disappeared, and WHO-FC had decreased to Ⅲ (Figure 4). At the follow-up assessment conducted 4 mo later, his WHO-FC was Ⅲ and the plasma BNP level had decreased to 35 pg/mL. Echocardiography revealed that TRPG had decreased to 56.2 mmHg, the degree of interventricular septal deviation toward the left ventricle had decreased, and pericardial effusion was absent (Figure 3B). Cardiac magnetic resonance imaging-derived RVEF improved to 50.8%, and RHC revealed improvement in mean PAP (34 mmHg), CI (2.9 L/min per square), and PVR (4.7 Wood units).

Both patients gave their written informed consent prior to their inclusion in the present study. The present study complied with the Declaration of Helsinki.

DISCUSSION

In the latest guidelines for PH, the recommended treatment strategy for PoPH is similar to that for PAH, while liver transplantation is considered in a selected subset of patients[6]. Based on this strategy, intravenous epoprostenol would be considered for WHO-FC Ⅳ patients,

including the present two cases. However, the successful use of oral agents effective against PAH has been reported for PoPH patients[7-9], suggesting a potentially important role of these drugs in PoPH treatment. In the two cases presented here, the use of intravenous epoprostenol was initially considered. However, it could not be used because of comorbid thrombocytopenia, which was thought to increase the risk during Hickman catheter implantation[10], and the probable further deterioration of thrombocytopenia caused by intravenous epoprostenol treatment itself[11]. In addition, both patients had been diagnosed with hepatocellular carcinoma, which further supported the use of a conservative treatment strategy. Considering the severe and rapid progressive state of the disease, we initiated combination treatment using ambrisentan, a dual endothelin receptor antagonist with limited liver toxicity, and tadalafil, a long-acting phosphodiesterase 5 inhibitor.

After the oral combination therapy, the patients' signs and symptoms dramatically improved. In addition, pulmonary hemodynamics improved. In particular, PVR decreased by 41% in case 1 and by 75% in case 2. This degree of PVR reduction was comparable with or greater than that achieved by drugs effective against PAH[12]. Furthermore, WHO-FC, the plasma BNP level, and CI notably improved after treatment in both cases in the present study, suggesting a favorable clinical outcome[13].

Notably, some clinical features were unique to the two cases of PoPH presented here. One was the rapid progression of disease. Pathologically, PoPH cases reportedly show vascular remodeling, as observed in idiopathic PAH[10,11], which is likely to develop gradually. However, in these two cases, PH-related symptoms and signs rapidly progressed during the month prior to admission. In addition, CI is usually increased in PoPH, reflecting the portal systemic shunt. However, in the present two cases, CI decreased. One possible interpretation of these clinical features is a unique pathogenesis of PoPH in these two cases, such as vascular spasm, rather than the gradual progression of pulmonary vasculopathy typically observed in PoPH[14,15]. Such a rapid pathogenesis may explain why oral treatment dramatically improved the clinical features in the short term. In addition, decreased cardiac function, as represented by CI, may have been caused by an unusually rapid elevation of PAP/PVR, which quickly resolved after treatment.

In the Reveal registry, the survival rate of PoPH is reportedly worse than that of idiopathic PAH and familial PAH[16]. Two possible explanations have been proposed for the worse clinical outcome of PoPH. First, comorbid advanced liver diseases such as liver cirrhosis and cancer can negatively impact survival. Second, comorbid liver disease and its complications also impede the optimal use of drugs effective against PAH, such as endothelin receptor antagonists and intravenous epoprostenol. In the present two cases, long-term outcome must be evaluated, despite the short-term outcome (up to 5 mo) being favorable.

In conclusion, we presented two cases of severe PoPH with a favorable clinical response to a combination of ambrisentan and tadalafil. Although this approach cannot be generalized, this combination therapy should be considered in selected patients with severe and rapidly progressive PoPH. Further studies would be required to better understand the pathogenesis and establish optimal treatment strategies for PoPH patients.

COMMENTS

Case characteristics

Two male patients with liver cirrhosis and portal hypertension.

Clinical diagnosis

Rapidly progressive exertional dyspnea.

Differential diagnosis

Progression of liver dysfunction, pulmonary and cardiovascular disease.

Laboratory diagnosis

Case 1: thrombocytopenia (5.0 x 10^4/mL), and increased D-dimer (9.38 μg/dL), transaminases, and plasma BNP levels (2212 pg/mL); Case 2: thrombocytopenia (3.5 × 10^4/mL), and increased D-dimer (4.39 μg/dL) and plasma BNP levels (988 pg/mL).

Imaging diagnosis

Right ventricular dilatation and increase in the estimated systolic pulmonary artery pressure by transthoracic echocardiography in both cases. Abdominal CT scan revealed findings of portal hypertension, whereas ventilation/perfusion lung scintigraphy showed no significant mismatch in both cases.

Treatment

Both patients were treated with ambrisentan–tadalafil combination therapy for rapidly progressive portopulmonary hypertension (PoPH).

Related reports

Successful monotherapy using an oral agent effective against pulmonary artery hypertension has been recently reported for patients with PoPH.

Term explanation

PoPH is a subtype of pulmonary hypertension (defined as a mean pulmonary artery pressure equal to or greater than 25 mmHg) that develops in patients with portal hypertension.

Experiences and lessons

A combination of ambrisentan and tadalafil may be a safe and effective therapeutic option for a certain subset of patients with PoPH and advanced thrombocytopenia.

Peer review

It is a good paper for publication.

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5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

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10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

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Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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